CONTROL OF CELLULAR DIVISION AND DEVELOPMENT

Dennis D. Cunningham, Eugene Goldwasser and James Watson, Organizers

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Control of Animal Cell Proliferation

286 CONTROL OF ANIMAL CELL PROLIFERATION, Robert W. Holley, The Salk Institute, San Diego, CA 92138

Work in many laboratories has indicated that the mechanisms that control growth are complex. Many different types of materials have been found to initiate growth, when they are added to the culture medium of quiescent cells. Synergisms between different growth-controlling factors are often observed.

The growth-stimulating factors that are probably of greatest importance *in vivo* are the polypeptide growth factors, including epidermal growth factor (EGF) and fibroblast growth factor (FGF). These factors stimulate growth at ng/ml concentrations. Present information suggests that there are additional, unidentified polypeptide growth factors.

At least two growth-controlling influences outside the cell remain poorly understood. One is the importance of endogenous growth inhibitors. The other is the role of cell surface area and/or shape.

The existence of endogenous growth inhibitors has been suggested over many years. Recently we have isolated a growth inhibitor, produced by kidney epithelial cell cultures, that appears to be a polypeptide with a molecular weight of approximately 15,000. It is active as a growth inhibitor at ng/ml concentrations, making it as active as a growth inhibitor as the polypeptide growth factors are as growth-stimulators. To the extent it has been tested, its growth inhibitory action has been found to be specific for kidney epithelial cells. These results support the view that growth inhibitors will turn out to be an important part of the growth control system *in vivo*.

The role of cell surface area and/or shape will be discussed.

The polypeptide growth factors act by binding to specific cell surface receptor sites. These interactions, which are being studied in many laboratories, lead to a series of events inside the cell that culminates in the initiation of DNA synthesis. There are two different simple models of the initiation of DNA synthesis. The growth-controlling factors may act directly to influence the processes that control the initiation of DNA synthesis. Alternatively, the growth-controlling factors may act on general cellular processes, including protein synthesis, which in turn lead to the initiation of DNA synthesis. More complicated models will also be discussed.

Control of Cellular Division and Development-Overviews of the Cell Systems

287 CONTROL OF HEMATOPOIETIC CELL PROLIFERATION AND DIFFERENTIATION IN VITRO, Donald Metcalf, Antony W. Burgess, Nicos A. Nicola and Gregory R. Johnson, The Walter and Eliza Hall Institute of Medical Research, P.O., Royal Melbourne Hospital, Victoria 3050, Australia.

The control of proliferation and differentiation of normal and leukemic hematopoietic cells can be analyzed in semi-solid cloning systems that are now available for all major hematopoietic populations (1).

In the best-studied system, colonies of up to 5000 granulocytes and/or macrophages can be grown from specific progenitor (colony-forming) cells (GM-CFC). Colonies can be grown in serum-free medium and from single micromanipulated cells. Cell division is entirely dependent on stimulation by a specific glycoprotein, granulocyte-macrophage colony-stimulating factor (GM-CSF) that has been purified and is active at 10^{-11}M concentrations. GM-CSF has concentration-dependent effects on cell cycle status, cycle times and the differentiation of GM cells. Two major molecular forms of GM-CSF have been identified. With the 23,000 MW form, high concentrations selectively stimulate granulocyte formation, low concentrations macrophage formation.

GM-CSF can also stimulate the initial, but not the terminal, cell divisions of multipotential hematopoietic cells and cells forming erythroid, eosinophil and megakaryocyte colonies. This cell proliferation is achieved without altering the pathway of differentiation of the responding cells (2).

Low molecular weight tissue-derived factors have been described that have a reversible, non-toxic, inhibitory effect on GM proliferation, possibly by reducing responsiveness to GM-CSF.

Myeloid leukemic cells retain responsiveness to the differentiating effects of GM-CSF (3,4) and in man remain fully dependent on GM-CSF for proliferative stimulation.

Analogous glycoproteins regulate erythroid, eosinophil and megakaryocyte proliferation in vitro and these closely similar molecules may constitute a family of regulatory molecules of common phylogenetic origin.

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288 NORMAL AND NEOPLASTIC MATURATION OF LYMPHOCYTES, I.L. Weissman, Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305

The function of the lymphoid system is to provide a surveillance mechanism for recognition and response to antigen-bearing molecules and cells. To carry out these functions the system includes a large number of interacting subsets of T cells, B cells, and other hematolymphoid cells, all with cell membrane markers enabling their interactions. Immune recognition takes place in specialized antigen-draining organs, and the first consequences of antigen recognition is to signal clonal lymphocyte division by triggering a receptor-mitogen complex. In this paper I will present some aspects of stage-specific lymphocyte development, focussing on cell membrane markers involved in maturation and in various recognitive functions. My main model will be normal T cell development in the thymus, selection of thymic lymphocyte subsets via cell-cell interactions, and the development of various T cell receptor systems necessary for tissue homing, lymphocyte interactions, and antigen recognition. The final subject will be the consequences of uncontrolled signalling of the T cell receptor-mitogen complex-lymphomagenesis.

289 PROPERTIES OF SARCOMA GROWTH FACTOR AND OTHER PEPTIDE HORMONES PRODUCED BY MOUSE AND HUMAN TUMOR CELLS, George J. Todaro and Joseph E. De Larco, Laboratory of Viral Carcinogenesis, National Cancer Institute, National Inst. of Health, Bethesda, Md. 20205

Mouse 3T3 cells have been transformed in culture by a variety of agents including DNA-containing viruses (SV40, polyoma), RNA-containing viruses (e.g. MSV, avian sarcoma viruses) as well as by various chemical carcinogens and radiation. Of these, the clones transformed by mouse sarcoma viruses specifically demonstrate a loss of cell surface epidermal growth factor (EGF) receptors. The basis for this apparent loss of receptors is shown to be the production by the MSV-transformed cells of a low molecular weight peptide (9-11K) related to EGF. This sarcoma growth factor (SGF) stimulates normal fibroblasts to assume a "transformed" morphology in monolayer cultures and to form progressively growing colonies in soft agar. EGF, under similar conditions, stimulates cell division in monolayer cultures but not anchorage independent growth in semi-solid medium.

SGF has been purified from serum-free medium of MSV-transformed 3T3 cells. A critical step in the purification involves the use of formalin-fixed human carcinoma cells, designated A431, that have exceptionally high levels of EGF receptors (2.2 x 10^6 receptors/cell). The sarcoma growth factor will bind to the receptors at neutral pH and elute from them in weak acid with the biologic activity (induction of anchorage independent growth, stimulation of cell division, specific binding to EGF receptors) retained. Cells lacking EGF receptors are unable to respond to either EGF or SGF. The two growth factors differ from one another in several ways. Antisera to EGF does not immunoprecipitate SGF and antisera to SGF does not immunoprecipitate mouse EGF. By isoelectric focusing EGF has a pI of 4.4, while SGF has a pI of 6.8. They also migrate differently from one another on polyacrylamide gradient gels. Most importantly, SGF acts in many ways as a "transforming" protein while EGF does not.

Certain human tumor cells have been found that produce a peptide growth stimulating factor that is similar in many respects to SGF. A factor produced by a human rhabdomyosarcoma cell line, A673, has been partially purified. It has an apparent molecular weight of 21,000 and competes with EGF for EGF specific membrane receptors. Like mouse SGF, it induces normal cells, of either rodent or primate origin, to proliferate in soft agar and it also brings about a rapid morphologic transformation of cells in monolayers. Binding to and eluting from the EGF receptor-rich human carcinoma cells, A431, results in substantial purification of this growth factor. The exact relationships between human SGF and mouse SGF remain to be determined. The possibility will be considered that SGF is a more "virulent" form of a normal growth regulatory protein.

Developmental Events Arising from Gene Expression or Modification

290 STRUCTURE AND EVOLUTION OF GENES, Walter Gilbert, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge MA 02138

Genes in higher cells contain noncontiguous coding regions, exons, interspersed with other DNA, introns. I shall discuss the hypothesis that the major role of introns is to separate the exons so that they will evolve more rapidly and to encourage their shuffling to make new genes. The evolution of the genes for insulin provide an interesting test case.

I shall discuss the mechanism of splicing, and to what extent an RNA molecule, a guide RNA, may play a role in the processing of messenger RNA.

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2) Church, Slonimski and Gilbert, "Pleiotropic Mutations within Two Yeast Mitochondrial Cytochrome Genes Block mRNA Processing" Cell, December 1979.

291 THE T/t-COMPLEX IN THE MOUSE: MUTATIONS THAT IMPAIR DIFFERENTIATION, Dorothea Bennett, Laboratory of Developmental Genetics, Sloan-Kettering Institute for Cancer Research, New York, N.Y. 10021

The T/t-complex in the mouse contains multiple genetic factors, capable of independent mutation and separable by recombination, that affect specific events of differentiation during embryonic development and spermatogenesis. Morphological and serological (1), as well as biochemical (2) studies have suggested that t-mutations are associated directly or indirectly with abnormalities of the cell surface. Recent efforts to identify the molecular nature of these abnormalities gives evidence for at least two different types of molecules associated with complex lethal t-haplotypes. One molecule, a non-glycosylated protein of 63,000 Daltons, is a direct gene product specified in apparently the same mutant form by each of over 30 independent t-haplotypes examined that contain the mutant gene responsible for tail interaction (3). On the other hand, the serologically defined antigenic determinants that define specific lethal t-haplotypes have been shown to reside on different oligosaccharides (C. Cheng, in preparation). The implication of these data with respect to the arrangement and function of mutant factors within the T/t-complex will be discussed.

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292 GENETIC ANALYSIS IN SOMATIC MAMMALIAN CELLS, Theodore T. Puck, Eleanor Roosevelt Cancer Research Institute, University of Colorado Health Science Center, Denver, Colorado 80262.

Mutant clone production, hybrid formation, cytogenetic analysis, and biochemical studies can be combined to afford insights into mammalian cell behavior. Mutants with a variety of properties have been prepared including resistance to lethal conditions; lack of specific biosynthetic enzymes leading to auxotrophy;loss of the regulatory system controlling biosynthesis of a specific metabolite like cholesterol; increased sensitivity to lethal agents like ultra violet radiation; presence or absence of specific cell-surface antigens; specific t-RNA deficiencies; deletions of particular chromosomal regions; changes in individual components of a multifunctional enzyme complex; and altered regulatory responses to metabolites,drugs, hormones, and cAMP-related reagents. Such mutants permit production of hybrids retaining limited numbers of human chromosomes. They make possible regional and fine-structure mapping of human genes, metabolic analysis of human disease situations, and study of altered patterns of cyclic AMP actions in different cell and hybrid clones.

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Effector Molecules which Regulate Cellular Development and Division

293 THE ROLE OF COLONY STIMULATING FACTOR IN GRANULOPOIESIS, Richard K. Shadduck, Giuseppe Pigoli and Abdul Waheed, Department of Medicine, University of Pittsburgh School of Medicine, Montefiore Hospital, Pittsburgh, PA 15213

The proliferation and maturation of granulocytic-monocytic stem cells appears to be controlled by a series of closely related glycoproteins termed "colony stimulating factors" (CSF's). Incubation of bone marrow cells with CSF in semi-solid gels leads to the growth of individual colonies composed of mature granulocytes and monocyte-macrophages. Correlative studies have shown increased serum levels of CSF in response to neutropenia. However, injection of impure CSF is only associated with modest stimulation of granulopoiesis. Since in vivo effects on granulopoiesis may be due to contamination with endotoxin or foreign proteins, definitive studies require a homogeneous source of CSF. Recently, we devised a six-step scheme for the purification of murine fibroblast (L-cell) derived CSF. Ten liter pools of conditioned media were concentrated by ultrafiltration, precipitated by ethanol and separated on DEAE cellulose, Con-A Sepharose and Sephadex G150. The CSF was separated from trace contaminants, including endotoxin, by density gradient centrifugation. The purified material has been radioiodinated and used to define the serum half life and in vivo distribution. Following IV injection there was a biphasic serum clearance with $T_{½}$'s of 24-40 minutes and 2-2½ hours in the first and second phases. Approximately 25% of the tracer was excreted in the urine at 6 hours; however, urinary radioactivity was due to low molecular weight peptides. Simultaneous studies with unlabelled CSF showed a similar rapid clearance from the serum but virtually no urinary CSF activity. Thus, assays for urinary CSF may not provide useful measures of in vivo CSF activity. In these short term experiments, less than 2% of the labelled tracer was bound to marrow cells whereas >40% was detected in the liver. Further in vitro studies have defined the interaction of CSF with responsive cells in the marrow. Varying doses of CSF were incubated with 10^7 marrow cells for intervals of 24-48 hours. The major increment in cell-associated radioactivity occurred between 6 and 16 hours. The reaction was saturable with 400,000 cpm or 50-80 units of CSF. Binding was prevented by cold CSF, but not by other proteins. Only minimal binding was noted with peritoneal macrophages or peripheral blood leukocytes. Irradiation or incubation with cytosine arabinoside, vincristine, mitomycin or colcemid yielded only a minimal reduction in CSF binding. This reaction appeared to require protein, and thereby new receptor, synthesis as binding was completely inhibited by cycloheximide (1 mcg/ml) and puromycin (10 mcg/ml). Antibodies to CSF were markedly inhibitory to granulopoiesis in peritoneal diffusion chambers in irradiated mice but did not reduce cellular differentiation of intact mice. These differences may be due to accelerated clearance of injected CSF in non-irradiated mice or to extensive stromal interactions which modulate and perhaps control granulocytic differentiation in the intact bone marrow microenvironment.

294 THYMOPOIETIN-IMMUNOREGULATION & INDUCTION OF DIFFERENTIATION BY THE SAME MOLECULE. Gideon Goldstein, Ortho Pharm.Corp., Raritan, N.J.08869 and Catherine Y. Lau, Ortho Pharm Corp., Don Mills, Ontario, Canada M3C1L9.

Thymopoietin is a 49 amino acid polypeptide hormone isolated from thymus.[1,2] The validity of its sequence was confirmed by synthesizing a pentapeptide corresponding to residues 32-36(TP5) and this peptide has the biological activity of thymopoietin itself.[3]

Study of the biological activity of thymopoietin has passed through several phases:

1/ Thymopoietin was isolated by its effect on neuromuscular transmission, an effect detected in studies related to the human disease myasthenia gravis. Thymopoietin induces a slight decrease in neuromuscular transmission and this effect appears twenty-four hours after administration.[1]

2/ Thymopoietin induces selective prothymocyte to thymocyte differentiation and inhibits early B cell differentiative steps.[4]

3/ Thymopoietin affects peripheral T cells and probably other components of the immune system. Its actions are hermeostatic and dose dependent, with enhancement of sub-optimal immune responses at lower doses and supporession of optimal immune responses at higher doses.

Preliminary studies with thymopoietin in various animal models indicate that, under various circumstances, it affects the immune system in different ways.

1/ Certain parameters of the immune response, which are declining in old mice, are restored by TP5 injections.[5]

2/ In a model of autoantibody induction in mice, autoantibodies are suppressed by TP5 injections.

3/ In models of experimentally transplanted tumors in mice, tumor growth and metastasis is inhibited by TP5.

These findings suggest that thymopoietin's immunoregulatory actions may be utilizable in human therapy.

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295 PURIFICATION AND PROPERTIES OF T-CELL REPLACING FACTORS, James Watson and Diane Mochizuki, Department of Microbiology, University of California, Irvine CA 92717

The induction of immune responses requires an interaction between lymphocyte and macrophages. Communication between lymphocytes involves humoral mediators. The purification and biological properties of a mediator that stimulates immune responses in T cell-depleted murine spleen cultures will be described. The mediator, termed Interleukin II, has the following biological activity: (1) stimulates continuous growth of T cell lines in culture; (2) potentiates the mitogenic response of thymocytes to plant lectins; (3) stimulates the antibody response to heterologous erythrocyte antigens in T cell-depleted spleen cultures; and (4) generates cytotoxic T lymphocytes in thymocyte as well as athymic spleen cultures. The assay systems in conjunction with standard biochemical techniques permit the quantitation and purification of Interleukin II. The purification of Interleukin II from murine, rat, and human sources involves concentration by salt precipitation or vacuum dialysis, purification on ion-exchange chromatography, gel filtration, preparative isoelectric focusing and elution from polyacrylamide gels. Gel filtration analysis indicates that murine Interleukin II activity is present in molecules of the 30,000 daltons molecular weight range. Activity is associated with molecules with isoelectric points of 4.3 and 4.9. Analysis of ^{125}I-labeled, IEF-purified Interleukin II in SDS-polyacrylamide slab gels reveals multiple polypeptides. Elution of factor activity from polyacrylamide gels is the definitive purification step which will permit the assignment of biological activities to a specific factor(s). Murine, rat, and human Interleukin II are antigen-dependent in its mode of action in that only antigen- or mitogen-activated murine T cells are rendered responsive to activity. Interleukin II appears to act as a hormone in stimulating the clonal expansion and differentiation of activated T cells. It exhibits an apparent lack of specificity with respect to immune response or antigen being studied as would be expected with a molecule of hormone-like activity. Evidence will be presented that activated T cells express surface receptors for Interleukin II. Purification to homogeneity will define the specific effector molecules involved in a particular biological activity. In addition, purification will facilitate studies elucidating the biochemical nature of factor as well as defining the target cell(s) and physiologic mode of action.

296 THE PLATELET DERIVED GROWTH FACTOR - ITS EFFECTS ON CELLS, Russell Ross, Elaine W. Raines, Beverly K. Kariya-Jones, Andreas Habenicht, Alan Chait, Arthur M. Vogel, John A. Glomset and Edwin L. Bierman, University of Washington School of Medicine, Seattle, Washington 98195

The platelet derived growth factor (PDGF), a factor present in the alpha granule of the platelet, has been purified from frozen-thawed outdated human platelet rich plasma in a series of six chromatographic steps. These are in sequence: CM-Sephadex (eluted with 0.1 M $(NH_4)_2CO_3$, pH 8.9); Biogel P-150 (1.0 N HAc); DEAE-Sephadex (0.05 M Tris, pH 8.9); Phenyl-Sepharose, (eluted with 2.0 M guanidine HCl in 0.05 M Tris, pH 8.9); Biogel P-60 (1.0 N HAc), activity peak 25,000-28,000; and High Pressure Liquid Chromatography (Waters I-125 silica based protein-sizing (1.0 N HAc). The factor has been purified approximately 50,000 times. PDGF acts in a coordinate fashion with components in plasma to initiate DNA synthesis and multiple cell doublings in cells such as 3T3, fibroblasts, smooth muscle and glial cells - but has no mitogenic effect upon endothelium. Within 3 hours after exposure to PDGF cells are also stimulated to increased bulk phase pinocytosis, increased binding of low density lipoprotein to high affinity cell surface receptors and to increased cholesterol synthesis in the absence of an external source of cholesterol. Using inhibitors of cholesterol synthesis (25-hydroxycholesterol or compactin) it can be shown that PDGF stimulation of cholesterol synthesis is a separable event and is not necessary for the stimulation of DNA synthesis by PDGF. This factor exerts multiple effects upon susceptible cells that are associated with increased metabolic activity and cell proliferation. Supported in part by grants from the USPHS HL-18645, AM-13970 and RR-00166.

Growth Factors for Anchorage-Dependent Cells

297 GROWTH FACTORS AND GROWTH REGULATION OF ANCHORAGE DEPENDENT CELLS,
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The growth of fibroblastic cells in tissue culture depends on external conditions, principally appropriate substratum, growth factors, nearby cells, and nutrients. Non-tumor forming cells are arrested with G1 DNA content when these conditions are suboptimal. Transformation to tumorigenicity in vivo is generally accompanied by a relaxation of the conditions for culture growth. Different transforming agents relax the growth requirements to different degrees. Dimished growth factor requirements of transformed cells have been investigated (1), particularly with a totally defined medium (2). A diminished EGF requirement was most closely correlated with transformation. Our proposal that ability of a cell to carry out a cell cycle and divide depends on whether external conditions permit a biochemical process in the earlier part of G1 phase was further investigated. Concentrations of cycloheximide that inhibit protein synthesis by about 50% specifically lengthen the part of G1 lying up to about 2 hr before S phase (3). A model that quantitatively accounts for the results is based on the proposal that a cell must synthesize a labile protein in adequate amount before it can proceed toward DNA sythesis. Cells transformed by several agents - DNA virus, RNA viruses, Chemicals, or spontaneously do not exhibit lengthened early G1 periods in the presence of low cycloheximide concentrations. Other data bearing on the labile protein hypothesis of growth control will be presented.

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298 EFFECT OF GROWTH FACTORS ON CELL CYCLE EVENTS.
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The platelet-derived growth factor (PDGF), which is found in serum but not in plasma[1], has been purified to homogeneity[2,3]; it stimulates replication at a concentration of 10^{-10}M[2,3]. Brief treatment with PDGF causes density-inhibited Balb/c-3T3 cells to become competent to synthesize DNA[4]; pituitary fibroblast growth factor (FGF) or precipitates of calcium phosphate also induce competence[5]. Continuous treatment with plasma allows competent, but not incompetent, cells to synthesize DNA[4]. A critical component of plasma is somatomedin, a group of hormones with insulin-like activity; multiplication-stimulating activity (MSA) or insulin replace plasma somatomedin in promoting DNA synthesis[5].

We have studied the molecular correlates of competence and the role of SV40 gene A products in regulating DNA synthesis. Treatment of quiescent cells with pure PDGF or FGF causes the preferential synthesis of five cytoplasmic proteins (approximate M.W. 20K, 29K, 42K, 60K, and 72K detected by SDS-PAGE under reducing conditions). Two of these competence-associated proteins (20K and 29K) are found within 40 min of PDGF addition; they are not induced by plasma, insulin, or epidermal growth factor (EGF). PDGF, FGF, or calcium phosphate induce a morphological change, the transient deciliation of the centriole, detectable by immunofluorescence, within 2 hr. Plasma, EGF, or MSA do not regulate deciliation. SV40 induces DNA synthesis in growth-arrested cells, but does not cause this transient centriole deciliation.

Gene A variants of SV40, including a mutant with temperature sensitive (ts) T-antigen (ts A209), a deletion in t-antigen (dl 884) and several ts A209 strains containing t-antigen deletions were used to induce DNA synthesis in Balb/c-3T3 cells. Like wild type SV40, all strains induced DNA synthesis equally well under permissive or non-permissive conditions. Addition of PDGF or plasma had little effect on SV40-induced DNA synthesis. Thus, the viral function that induces DNA synthesis is not t and is not temperature sensitive. This gene function overrides the cellular requirement for hormonal growth factors. It does not induce transient centriole deciliation, a hormonally-regulated event.

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Cell Surface Receptors Involved in Mitogenesis and Development

299 PROPERTIES OF RECEPTORS FOR EGF AND THE COAT ANTIGEN (gp70) OF RAUSCHER MURINE LEUKEMIA VIRUS,

C. Fred Fox, Peter S. Linsley, Ken Iwata and Beate Landen, Molecular Biology Institute and Department of Microbiology, University of California, Los Angeles, CA 90024

Treatment of cell membranes with nonionic detergent renders approximately half the EGF binding activity operationally "soluble". The other half readily sediments at low centrifugal force. Both the solubilized and sedimentable forms of receptor form the "direct linkage" complex in which EGF becomes covalently bound to receptor (1). The soluble and sedimentable forms of receptor also had similar K_D's for EGF binding, but differed dramatically in their response to EGF in the membrane protein phosphorylation assay described by Carpenter et al. (2). The easily sedimentable form of the receptor is totally refractory to EGF-induced phosphorylation when measured after treatment with nonionic detergent and separation of the sedimentable and soluble fractions. The soluble fraction, on the other hand, had all the EGF-induced protein kinase activity, and the only major proteins phosphorylated were also acceptors for EGF in the "direct linkage" reaction. This indicates that EGF receptor proteins are the major phosphate acceptors. When membranes were treated with EGF and gamma-Pi^{32}-ATP, before detergent addition, a significant fraction of the labeled receptor protein appeared in the pellet fraction. This indicates that the kinase activity and receptor protein are separable, or that EGF-induced phosphorylation promotes receptor aggregation. Gel filtration and sedimentation analyses of the detergent soluble EGF binding components reveal a heterogeneous array of supramolecular aggregates which bind EGF, while little EGF binding activity appears as a monodisperse entity. These data show that EGF receptors exist largely as aggregates on the membrane prior to EGF binding and may explain the ability of other polypeptide hormones (FGF, PDGF) to "down regulate" EGF receptors (3).

We have recently purified a receptor for gp70, the coat antigen of Rauscher murine leukemia virus (4-6). Unlike the EGF receptor, this protein is not internalized by cells, but is shed into the culture medium. Preliminary studies on the biological properties of this protein will be presented.

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300 ANALYSIS OF CELL SURFACE COMPONENTS IN THROMBIN-STIMULATED CELL DIVISION,

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Highly purified thrombin (Th) can stimulate division of certain nonproliferating cultured fibroblasts under serum-free conditions (1). The stimulation can be produced by Th action at the cell surface (2). There are specific cell surface receptors for Th to which it must bind to initiate cell proliferation (3). The stimulation requires the proteolytic activity of Th since Th inactivated by derivatizing its catalytic site serine with diisopropylphosphate binds to receptors as well as active Th but does not stimulate cell division. These results prompted us to look for specific cell surface cleavages by Th required for stimulation of cell division. One required cleavage involves a 43K dalton component that appears to be the Th receptor; this component was present on responsive and four unresponsive populations of chick embryo cells, but was cleaved by Th only on the populations that responded to its mitogenic action (4). To permit a detailed evaluation of this component and a search for other cell surface cleavages that might be required for the stimulation, we have applied procedures to maximize the resolution of Th-sensitive cell surface components. These include the separation on two-dimensional gels of components labeled by $^{125}I^-$ and lactoperoxidase, ^{35}S-methionine, ^3H-fucose, or ^3H-borohydride after neuraminidase and galactose oxidase treatment. The experiments were conducted on a line of Chinese hamster lung cells from which it is possible to obtain cloned populations of cells that are responsive or unresponsive to the mitogenic action of Th. With responsive cells, we found a 43K dalton Th-sensitive cell surface component apparently similar to the one on chick embryo cells. It had a pI distinct from actin and was metabolically labeled with ^{35}S-methionine. We also found Th-sensitive cell surface components of about 25K, 35K, and 250K daltons on responsive cells. These studies also revealed the appearance of a 30K dalton component with a pI of about 6 after Th treatment of responsive cells that appears to represent a cleavage product. We plan to examine these cleavages in a series of unresponsive clones to determine if they are necessary for Th-stimulated cell division. We will also examine other Th-mediated changes in these clones such as the direct covalent linkage of Th to its cell surface receptors (5). (Supported by NIH Grant CA-12306).

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301 INSULIN RECEPTOR SYNTHESIS AND TURNOVER IN DIFFERENTIATING 3T3-L1 PREADIPOCYTES,
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3T3-L1 preadipocytes can be induced to differentiate in culture into cells with characteristics of adipocytes. ^{125}I-Insulin binding to 3T3-L1 cells was compared to that of non-differentiating 3T3-C2 controls (1). In the absence of inducing agents, e.g. insulin, methylisobutylxanthine and dexamethasone, 3T3-L1 cells fail to express the adipocyte phenotype and maintain ~30,000 insulin binding sites/cell. Induction of 3T3-L1 cells with insulin present causes initial "down regulation" of insulin receptors followed by a 12-fold increase paralleling differentiation. 170,000 insulin binding sites/cell are expressed when >75% of the cells have differentiated. The rise of insulin receptor level is differentiation-dependent. 3T3-C2 cells which do not differentiate exhibit only insulin-induced receptor "down-regulation". The binding capacity of 3T3-L1 cells for epidermal growth factor is independent of differentiation state.

A density-shift technique was used to analyze insulin receptor synthesis and turnover in cells labeled with heavy (^{2}H, ^{13}C, and ^{15}N) amino acids (2). Solubilized newly-synthesized "heavy" and old "light" receptors were separated by isopycnic banding on CsCl gradients and quantitated. The differentiation-linked rise of soluble insulin receptor level by the isopycnic banding method paralleled the rise in receptor level by insulin binding to intact cells. Mixing experiments with "light" and "heavy" receptor prior to gradient separation revealed no change in their respective peak densities. Thus, no significant interchange of receptor subunit mass occurred. Receptor synthesis and turnover studied by the density-shift method showed that the rise in insulin receptor level during differentiation resulted primarily from an increased rate of receptor synthesis. Rate of insulin receptor degradation was not significantly altered. The $t_{1/2}$ (degradation) for the insulin receptor in differentiated 3T3-L1 cells in culture was 6.7 hours in the presence of insulin. Removal of insulin from the medium did not significantly affect receptor turnover.

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302 COLONY STIMULATING FACTOR AND THE REGULATION OF MACROPHAGE PRODUCTION.
E. R. Stanley, L. J. Guilbert, S. K. Das and L. Forman, Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York 10461.

Colony stimulating factors (CSFs) stimulate the proliferation and differentiation of granulocytes and macrophages and their precursors. A subclass of the CSFs that appears to be restricted in action to the stimulation of mononuclear phagocytic cells can be discriminated from other CSF subclasses by detection in a radioimmunoassay (RIA) (1) and a radioreceptor assay (RRA), as well as by physicochemical and biological properties. This subclass has been found in the circulation (1-5 ng/ml) of birds, rodents and man and has been purified from murine L cell conditioned medium (2) and human urine (3). CSF from both these sources is identical (4) to macrophage growth factor (5). The purified factor is a sialic acid containing glycoprotein comprised of 2 subunits of similar size and charge, associated by disulfide bonds (2). Subunits prepared by gentle reduction are devoid of activity determined by either bioassay, RIA or RRA. Of somewhat variable M_r (40K-80K), approximately 50% of the M_r of a 70K species can be attributed to carbohydrate that can be removed without loss of bioassay, RIA or RRA activity. It appears that the M_r variability encountered with this CSF subclass is in large part due to differing degrees of glycosylation.

The nature of the interaction of ^{125}I-L cell CSF (^{125}I-CSF) with murine peritoneal exudate macrophages has been studied (3,6). On incubation with 10 pM ^{125}I-CSF at 0°, specific binding to the adherent macrophages reaches a stable maximum within 15 h. Binding is saturated at 1 nM ^{125}I-CSF ($k_d \sim 11$ pM, $\sim 5 \times 10^4$ binding sites per cell) and is not competed for by other growth factors and hormones. The competition curves for L cell CSF and human urinary CSF are indistinguishable. By comparison with the 0° binding, at 37° the maximum degree of binding at 10 pM ^{125}I-CSF is lower and transitory, and a large proportion of the bound ^{125}I-CSF is destroyed by the cells. This destruction is best explained by rapid internalization followed by lysosomal degradation. Preliminary studies suggest that binding is restricted to macrophages and their precursors.

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Role of Major Histocompatibility Antigens in Cell-Cell Recognition

303 THE H-2 ANTIGEN AND CELL-CELL INTERACTIONS, Jan Klein, Max-Planck-Institute for Biology, Corrensstrasse 42, 7400 Tuebingen, F.R.G.
In contemporary vertebrates, the allograft reaction to antigens controlled by the major histocompatibility complex (MHC) is a nonphysiological phenomenon which is only mirroring the true MHC function; in the excitement generated by the discovery of physiological MHC function, the allograft reaction ceased to be a popular subject to study. Yet, allograft reaction remains a formidable clinical problem, the solution of which would revive the dying out art of organ transplantation. The time is, perhaps, ripe to have a new look at this old problem. In this presentation, I shall discuss recent results from my laboratory pertaining to three aspects of allograft reaction:
-- Genetic control of histocompatibility antigens;
-- complexity of the H antigens; and
-- cellular mechanisms involved in graft rejection.

304 STRUCTURAL STUDIES ON H-2 PRODUCTS FROM MOUSE MHC MUTANT STRAINS: IMPLICATIONS FOR CELL-CELL RECOGNITION, S.G. Nathenson, B.M. Ewenstein, J.M. Martinko, R. Nairn, T. Nisizawa, H. Uehara, K. Yamaga, Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York 10461.

The major histocompatibility complex (MHC) is a linked set of polymorphic genes whose products play crucial roles in the host reaction to antigenic stimulation. The classical K, D and L transplantation H-2 alloantigens are integral cell membrane glycoproteins of approximately 340 amino acids in length associated non-covalently with β_2-microglobulin.

Isolation, biochemical characterization and alignment of the CNBr fragments of the papain derived K^b glycoprotein has been accomplished, and the majority of the primary sequence determined (1,2,3). We have begun structural studies on the K and D products of a series of mouse haplotypes carrying H-2 MHC mutations in order to examine a genetic system in which specific structural alterations might be expected to be related to specific biological reactivity. Previous results have shown that, when compared to parental molecules, the dml mutant had about 30% differences in the peptide profile of its D protein (4), and bml and bm3 of the order of 10% differences of their K proteins (5). In order to approach the localization of such differences for the $H\text{-}2^b$ series, we carried out comparative analysis of the cyanogen bromide fragments from the K antigens of the bml, bm3 and bmll $H\text{-}2K^b$ MHC mutants. Peptide differences between the K glycoprotein of bm3 and that from the standard strain were localized in CNBr fragment Ib, and differences between the K molecule of bml and that of the standard strain were localized to CNBr fragment Ia. Initial studies suggest that two different amino acids have been altered in the Ib fragment of bm3, one at position 77 and the other at 89. The alteration in the bml mutant appears to be a single amino acid exchange at position 155. The K glycoprotein of a third mutant, bmll, which biologically shows similar but not identical reactivity to bm3, appears to have the change at position 77 but it does not have an alteration at 89. While these changes may not be the only differences between mutant and parent, these results suggest that for the K^b mutants examined so far one or two discrete amino acid alterations appear to be the predominant alteration in the mutant molecule. The observed changes are thus far localized to the 165 amino acids from the amino terminal end. This suggests that this part of the molecule may be particularly important for T-cell specificity in both allogeneic recognition and associated recognition, and further that relatively small discrete changes in amino acid sequence of the H-2 glycoprotein appear to have far-reaching affects on cellular interactions.

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Intracellular Events Following Triggering of Development or Division

305 MEDIATION OF ERYTHROPOIETIN ACTION, Eugene Goldwasser, Department of Biochemistry, University of Chicago, Chicago, IL. 60637.

Erythropoietin (epo) is the primary regulatory factor controlling red blood cell differentiation in normal mammals. It has been purified [1] to apparent homogeneity from urine of patients with hypoplastic anemia; is a glycoprotein with a molecular weight by SDS gel electrophoresis of 39,000 and consists of approximately 40% protein, the remainder most probably, being carbohydrate. Substitution of iodine (^{125}I) on the tyrosine residues causes inactiyation of epo as does the acylation of free amino groups, making it impossible, at present, to do direct experiments on the binding of epo to its target cells in marrow, or to determine whether epo is internalized. When rat marrow cells are exposed in vitro to epo the following molecular events occur in the temporal sequence listed: appearance of a cytoplasmic protein factor, (see below), transcription of HnRNA, transcription of rRNA, DNA replication, synthesis of band 3 red cell plasma membrane protein and two membrane glycoproteins, and synthesis of hemoglobin. The marrow cytoplasmic factor (MCF) is a protein formed after the interaction of epo with marrow cells, in the absence of protein synthesis, and is assayed by its effect on the stimulation of transcription by isolated nuclei. It is obtained only from epo treated marrow cells although it can affect nuclei from other tissues[2]. MCF affects isolated nuclei by increasing the activities of both α-amanitin resistant and sensitive RNA polymerases. It also has a small but significant effect on the template activity of the DNA in the nuclei[3]. We propose that MCF is the mediator substance that is formed as a result of the interaction of epo with specific receptors on the external surface of the target cell; it acts to convey the signal from the inner surface of the cell membrane to the nucleus. The subsequent transcriptive events including the formation of messengers for the different proteins to be made at specific times, are those that eventually define the emergent erythroid cells. The increase in DNA synthesis most likely represent the amplification divisions inherent in the system.

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306 EPIDERMAL GROWTH FACTOR--RECEPTOR--PROTEIN KINASE INTERACTIONS: SOLUBILIZATION AND PURIFICATION, Stanley Cohen, Department of Biochemistry, Vanderbilt University, Nashville, Tennessee 37232

Membranes were prepared from A-431 human epidermoid carcinoma cells which retained the ability to bind ^{125}I-labeled epidermal growth factor (EGF) in a specific manner. In the presence of [γ-^{32}P]ATP and Mn^{2+} or Mg^{2+}, this membrane preparation was capable of phosphorylating specific endogenous membrane proteins, as well as exogenously added histone. The binding of EGF to these membranes *in vitro* resulted in a several-fold stimulation of the phosphorylation reaction. The phosphorylation reaction was not dependent on cyclic AMP or cyclic GMP.

These findings suggested that one of the biochemical consequences of the binding of EGF to membranes is a rapid activation of a cyclic AMP-independent phosphorylating system.[1] The activation of the membrane associated protein kinase by EGF appears to be a reversible phenomenon.

The membrane preparation may be solubilized by a number of nonionic detergents with the retention of both ^{125}I-labeled EGF binding activity and EGF-enhanced phosphorylation of specific membrane proteins.

The solubilized membrane preparation may be purified by affinity chromatography. The purified preparation retains both EGF-binding activity and EGF-enhanced phosphorylation activity. Analysis of the affinity purified preparation by SDS gel electrophoresis indicates the presence of one major protein band of molecular weight 150,000 and several trace bands.

The evidence suggests that the major 150,000 protein band is the receptor for EGF and is a substrate of the phosphorylation reaction. The co-purification of EGF-binding activity and EGF-stimulated phosphorylation activity suggests an inherent close relationship.

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307 FACTORS CONTROLLING DIFFERENTIATED FUNCTION AND REPLICATION THROUGHOUT THE CULTURE LIFE SPAN OF ADRENOCORTICAL CELLS, Gordon N. Gill, Department of Medicine, University of California, San Diego, La Jolla, CA 92093

Bovine adrenocortical cell cultures have been developed for study of factors regulating differentiated function and replication of normal endocrine cells. Although homogeneous cell populations synthesize adrenal specific steroids and demonstrate a finite culture life span of ∿60 generations, changes occur in responsiveness to ACTH and in the pattern of steroids synthesized. Functional ACTH receptors are sensitive to cell density with decreases occurring as cell density is lowered; functional PGE_1 receptors do not vary under these conditions. Cytochrome $P\text{-}450_{11\beta}$, the final enzyme in the cortisol biosynthetic pathway, is diminished via a mechanism involving product-induced free radical generation. At lower O_2 tensions, and in the presence of antioxidants, P-450 is maintained and ACTH-induction of the enzyme is observed. FGF and angiotensin are principal growth factors for these cells; ACTH and other substances which increase cAMP production induce and stimulate differentiated function but inhibit replication. When both growth factors and cAMP-stimulating agents are added, a hypertrophied, hyperfunctional cell results. Cells are arrested at the G_1/S boundary until cAMP falls in response to desensitization to ACTH; limited replication then ensues. A model for interaction of growth and differentiated function stimulating factors is proposed.

Organization and Expression of Genes in Eucaryotic Systems

308 THE STRUCTURE AND ORGANIZATION OF ANTIBODY HEAVY CHAIN GENES, L. Hood, P. Early, M. Davis, H. Huang and K. Calame, Division of Biology, California Institute of Technology, Pasadena, CA 91125

DNA rearrangements of antibody gene segments play a fundamental role in two aspects of B-cell differentiation--diversification of variable genes and the switching of heavy chain classes. We will discuss the molecular nature of these two fundamental types of DNA rearrangements in the heavy chain gene family.

309 MOLECULAR STRUCTURE OF THE NATURAL OVALBUMIN AND OVOMUCOID GENES AND PRECURSORS TO THEIR MESSENGER RNAs. Bert W. O'Malley, Dept. of Cell Biology, Baylor College of Medicine, Houston, Texas, 77030.

The natural ovalbumin and ovomucoid genes were amplified by molecular cloning, and detailed analysis was accomplished by restriction enzyme mapping, Southern hybridization and electron-microscopic mapping and sequence analysis. Specific DNA probes prepared from structural and intervening sequences within the natural ovalbumin and ovomucoid genes were used to identify the high molecular weight precursors of these mRNAs. Using S_1 nuclease analysis, the 5'- and 3'- ends of the largest precursor for ovalbumin mRNA was mapped to the first and last nucleotides of the structural gene (i.e., the mRNA). Nuclear RNA from chick oviducts was electrophoresed under denaturing conditions (methylmercury hydroxide) and hybridized to ^{32}P-DNA probes for structural and intervening sequences. Hybridization of these probes to nuclear RNA demonstrated that multiple species of RNA exist which are higher in molecular weight than mature ovalbumin and ovomucoid mRNA. Pulse-chase experiments confirm a precursor-product role for conversion of the high molecular weight RNAs to their smaller biologically active products. These results are consistent with the entire ovalbumin and ovomucoid genes being transcribed into large precursor molecules, followed by excision and turnover of the intervening sequence RNA and consecutive ligation of the structural sequences to form mature ovalbumin and ovomucoid mRNAs. In addition to the natural ovalbumin gene, we have isolated from a chicken library two genes, designated X' and Y, which are linked to the ovalbumin gene in the order of 5'-X'-Y-ovalbumin-3'. Using Northern hybridization, RNA excess and DNA excess hybridization, we demonstrated that the Y gene is stimulated by the steroid hormone estrogen, but to a lower level than the authentic ovalbumin gene. In contrast, X'-gene transcription was not significantly affected by estrogen treatment. In studies directed towards replacement of the entire chicken ovalbumin gene back into eucaryotic cells, we cloned the natural genes together with flanking sequences and three copies of the Herpes Simplex Virus thymidine kinase gene in plasmid pBR322. This recombinant plasmid was linearized and used to transform thymidine kinase-deficient ($LMTK^-$) mouse cells. Thymidine kinase positive (TK^+) transformants were selected by their ability to grow in the hypoxanthine-aminopterin-thymidine (HAT) medium. The entire ovalbumin gene was integrated into high molecular weight cellular DNA within all the transformants and retained its original sequence organization. In all the TK^+ transformants examined, a protein identified as chicken ovalbumin by solid phase immunoassay was detected within the cells. It is estimated that between 1000-100,000 molecules of chicken ovalbumin was produced per mouse cell in each of these transformants. Our results demonstrate that the mouse cellular machinery can be utilized to accurately express genetic information encoded in a natural chicken gene (containing intervening sequences) into its specific protein product.

Intracellular Events Following Triggering of Development or Division

310 NERVE GROWTH FACTOR: RELATIONSHIP BETWEEN PLASMA MEMBRANE AND NUCLEAR RECEPTORS,

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Over the past several years, it has come to be appreciated that there are a variety of agents, of other than nutritional value, that specifically regulate the growth, development, and, in some cases, differentiation of cells both in vitro and in vivo. As such, they can be appropriately grouped with the "classical" hormones that have been variously designated as 'maintenance', 'developmental' or 'permissive' - a category that includes such substances as growth hormone, thyroid hormone and various steroid hormones (1) - albeit that in some instances their behavior, particularly with regard to transport, deviates from accepted definitions (2). The basis of this classification is primarily functional, although structural comparisons have revealed some common ancestries as well. To wit, these growth factors, which are for the most part polypeptides, transfer information to their target cells through specific complexation with plasma membrane receptor at the cell surface. In many cases, a stimulation of cellular metabolism ensues immediately that can be generally described as pleiotypic activation (3). Subsequently, with considerable variation in the time scale from one system to another, there develops a variety of long-term responses which may alter gene expression, stimulate cell division and/or produce marked alterations in cell morphology and behavior, all to the general benefit of the target tissue. Nerve growth factor (NGF) is one such molecule. It shows both short and long term effects in interactions with its responsive cells, i.e. sympathetic and selected sensory neurons, many of which are similar to those exerted by the other members of the insulin-related subset (insulin, insulin-like growth factor, relaxin and NGF). A plausible mechanism in which two classes of receptors, one type in the plasma membrane and one type in the nucleus, are responsible for the expression of the rapid and slow effects has been proposed (4). An internalization, apparently by endocytosis, links the two loci of action. Although the two receptor classes appear to be different as judged by binding and solubility characteristics, considerable structural similarity may well exist. The manner in which either receptor produces its effect after binding the hormone remains to be elucidated. Supported by NIH Grant NS 10229.

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311 GENETIC ANALYSIS OF STEROID HORMONE ACTION,

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The murine thymoma line W7 is highly sensitive to glucocorticoids and undergoes cytolysis in tissue culture in the presence of dexamethasone. Variants of W7 resistant to different concentrations of dexamethasone can be isolated. This system offers a genetic approach to study the mode of action of glucocorticoids on lymphoid cells, since the defects responsible for resistance in variants can be analyzed by biochemical and genetic means.

The parental dexamethasone-sensitive (Dex^S) W7 line contains approximately 30,000 dexamethasone binding sites per cell. These receptors have a binding constant for dexamethasone $Kd = 1.3 \pm 0.3 \times 10^{-8}M$, and 70% of the receptor-dexamethasone complexes are translocated to the nucleus. Variants resistant to high concentrations of dexamethasone (10^{-6} to $10^{-5}M$) have to be induced by various mutagenic treatments because their spontaneous frequency is $<10^{-9}$. All appear to have receptor defects, either having no detectable receptor (r^-) or a receptor defective in nuclear transfer (nt^-)[1].

In contrast, variants resistant to low concentrations of dexamethasone, in the range of 10^{-9} to $10^{-8}M$, arise spontaneously at frequencies of the order of 10^{-6}. The majority of these partially resistant variants contain approximately 15,000 dexamethasone receptor sites per cell, which display normal affinity and normal nuclear transfer. These results indicate that, while the parental W7 line is normally diploid for the receptor structural gene (r^+/r^+) its variants resistant to low dexamethasone concentrations are functionally hemizygous at that locus (r^+/r^-)[2].

The availability of cell lines containing either a diploid (r^+/r^+) or a haploid (r^+/r^-) amount of normal receptor allows an investigation of the correlation between intracellular receptor concentration and level of sensitivity. By fusion between homozygous (r^+/r^+ or r^-/r^-) and hemizygous (r^+/r^-) lines, hybrids have been constructed which contain one ($r^+/r^- \times r^-/r^-$), two ($r^+/r^- \times r^+/r^-$), three ($r^+/r^+ \times r^+/r^-$), or four ($r^+/r^+ \times r^+/r^+$) copies of the r^+ allele. Measurements using a whole-cell assay confirm that the receptor content of these hybrids reflects the r^+ gene dosage effect. The sensitivity of these hybrids estimated by their cytolytic response at various dexamethasone concentrations, increases with the concentration of receptors.

These results show that, in this model system, there is a tight correlation between the glucocorticoid receptor content and the level of sensitivity.

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Role of Substrate and Environmental Interactions in Growth and Development

312 THE CONTROL OF CELL PROLIFERATION BY THE EXTRACELLULAR MATRIX, Denis Gospodarowicz, Cancer Research Institute, Univ. of Calif., Medical Center, San Francisco, Ca., 94143

The extracellular matrix or basal lamina produced by cells is the natural substrate upon which cells migrate, proliferate and differentiate in vivo. It has been shown by others that extracellular matrices promote and stabilize the morphogenesis of the tissue associated with them. However, although numerous studies have dealt with the differentiation of tissue induced by the extracellular matrices, few studies have dealt with their effect on cell proliferation. Since in most organs cell proliferation precedes cell differentiation, it is likely that both proliferation and differentiation are controlled directly or indirectly by the substrate upon which the cells rest. We have therefore compared the proliferative behavior of four different cell types when maintained on plastic versus an extracellular matrix produced by corneal endothelial cells. We have also compared their responses to fibroblast growth factor (FGF) when they are maintained on either substrate. Bovine adrenal cortex cells, granulosa cells, and vascular or corneal endothelial cells maintained on plastic and exposed to optimal serum concentration do not proliferate unless they are exposed to FGF. In contrast, when maintained on a corneal extracellular matrix these same cell types proliferated actively and FGF was not required in order for the cultures to become confluent. One can therefore conclude that the close contact of the cells with the extracellular matrix must make them responsive to factors present in serum and that adherence to plastic prevents such a response. Therefore, those cells which, when maintained on plastic exhibit a total dependence on FGF, but not on serum, in order to proliferate, exhibit a total dependence on serum when maintained on an extracellular matrix and no longer require FGF. The simple change of substrate from plastic to extracellular matrix could therefore restore the sensitivity of the cells to mitogens present in serum, which could be the same as those present in plasma. To explore the possibility that the serum factors to which cells maintained on extracellular matrix become sensitive are also present in plasma, we have compared the mitogenic activity of plasma versus serum, using as target cells vascular smooth muscle cells maintained on either plastic or an extracellular matrix. When vascular smooth muscle cells are maintained on an extracellular matrix, they respond and proliferate as well in plasma as in serum. It is therefore likely that the serum factors to which cells respond when maintained on an extracellular matrix are the same as those present in plasma. Therefore, the previously observed differences between plasma and serum insofar as their growth-promoting activity is concerned are a function of the substrate upon which the cells rest. It is likely that plasma is as mitogenic as serum in vivo, since all cell types are in close contact with an extracellular matrix which is their natural substrate.

313 CELLULAR INTERACTIONS IN THE REGULATION OF HAEMATOPOIETIC STEM CELL PROLIFERATION AND DIFFERENTIATION T. Michael Dexter, Michel Lanotte, Elaine Spooncer, Deniz Toksoz, Paterson Laboratories, Christie Hospital, Withington, Manchester M20 9BX England

The proliferation of haemopoietic stem cells (CFU-S) *in vitro* depends upon a heterogeneous population of marrow derived adherent cells which we speculate provide the inductive environment essential for haemopoiesis (1). Within the adherent population several cell populations can be recognised - endothelial cells, fat cells, macrophages and fibroblast-like cells - which together form a complex multilayer. Within the adherent layer proliferation and differentiation of CFU-S is occurring. Proliferation is apparently determined by the production of cell cycle active stimulators and inhibitors of CFU-S. We speculate that production of stem cells is determined by modulation of the relative balance between stimulator and inhibitor. Differentiation of stem cells is not affected by the addition of relatively high concentrations of granulocyte/macrophage stimulatory molecules (GM-CSF) or erythropoietin (ep), provided that purified preparations are used. Impure preparations result in a rapid decline in haemopoiesis, presumably not mediated by the GM-CSF or epo. Antiserum raised against L-cell CSF does not inhibit granulopoiesis in long-term cultures (2). In recent work, we have been investigating the role of cell matrix components (particularly glycosaminoglycans) in defective versus competent adherent layers, and preliminary results will be reported. We have also been studying the potential of collagen gels to support prolonged haemopoiesis. Using such gels we have found that both haemopoietic and stromal elements can be induced to form colonies and that active haemopoiesis can be sustained for several weeks. Adherent cells appear to play an important role in this system. Preliminary results indicate that collagen gels can be useful for cloning the stromal elements involved and may, therefore, allow analysis of the cell type(s) important for the maintenance of stem cell proliferation, the cellular interactions involved, and the biochemical analysis of putative regulatory molecules produced. Results of this work will be presented.

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314 CELLULAR INTERACTIONS AND THE ENVIRONMENT IN LYMPHOCYTE DEVELOPMENT: THE ROLES OF ANTIGEN, HISTOCOMPATIBILITY AND GROWTH FACTORS IN T-CELL DEPENDENT B-CELL STIMULATION, Fritz Melchers[1], Jan Andersson[2], Waldemar Lernhardt[1], and Max H. Schreier[1]. [1]Basel Institute for Immunology, Basel, Switzerland; [2]Biomedicum, University of Uppsala, Sweden. T-cell dependent B-cell stimulation depends on cellular interactions between macrophages, T-cells and B-cells. T-cells recognize antigen in the context of Ia-determinants on macrophages. This leads to the activation of helper T-cells and to the production of helper factor(s) either by the activated helper T-cells or by the macrophages. Cloned lines of helper T-cells produce factors ("help") for B-cell replication and Ig-secretion in the presence of histocompatible macrophages and of specific antigen. These factors stimulate histocompatible as well as histoincompatible, mitogen-activated B-cell blasts polyclonally. Thus, neither antigen, nor histo-compatibility, but antigen-unspecific, soluble factors are required to stimulate an activated B-cell blast through the next round of division.

Small, resting B-cell, however, are not by these factors stimulated to replication, but only to polyclonal, H-2-unrestricted maturation to Ig-secreting cells. Replication (and Ig-secretion) of small resting B-cells, on the other hand, is only induced when antigen-specific small B-cell binds their specific antigen via surface Ig-molecules and interact with histocompatible helper T-cells. The preference of the resting B-cells for such collaboration with T-cell help is mapped to the K-end of the H-2 locus, and probable constitutes the antigen expressed on B-cells by the I-region. It appears that a resting B-cell cell is excited by the binding of specificantigen to surface Ig and by the interaction of its surface Ia with helper T-cells. After this dual recognition the excited B-cell can be stimulated by the antigen-unspecific factor(s) generated by the interaction of helper T-cells, adherent cells and antigen to initiate growth. Immune induction of a B-cell thus involves three controlling elements: Ig, Ia and B-cell growth factor receptors.

Teratocarcinomas

315 TERATOCARCINOMA CELLS AS VEHICLES FOR TRANSFER OF MARKER GENES INTO MICE, Beatrice Mintz, Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pa. 19111.

The stem cells of malignant mouse teratocarcinomas undergo a stable conversion to normalcy when they are microinjected into normal young embryos at the blastocyst stage (1). In this environment, cells from the tumor lineage can contribute to the differentiation of all somatic tissues and also occasionally to germ cells from which progeny are derived. The tumor stem cells thus provide a unique channel for the introduction of predetermined genetic markers into mice (2): During an initial period of in vitro culture, prior to injection into embryos, the cells are mutagenized and then specifically selected or screened to obtain cells with the mutation of interest. Examples are the isolation of cells with a gene-encoded deficiency in an enzyme such as hypoxanthine phosphoribosyltransferase (3) or in receptors such as those for low-density lipoprotein (4). Transfer of foreign cloned genes can also take place into cultured teratocarcinoma cells, as has previously been reported (5) for transfer of xenogeneic thymidine kinase and β-globin genes into cultured mouse fibroblasts. These options extend the possibilities of generating in vivo markers specifically designed for analyzing control of tissue-specific gene expression and for producing mouse models of human genetic diseases.

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316 EXPRESSION OF VIRAL GENES IN DIFFERENTIATED AND UNDIFFERENTIATED MURINETERATO-CARCINOMA CELLS, George Khoury and Shoshana Segal, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205

Undifferentiated murineteratocarcinoma cells do not support SV40 or polyoma virus replication or even the expression of the early papovavirus proteins. The F-9 line of murineteratocarcoma is particularly suitable for induction studies since it undergoes very limited differentiation under normal culture conditions. In earlier experiments we showed that undifferentiated F-9 murineteratocarcinoma cells infected with SV40 at high multiplicities (50-250 M.O.I.) synthesize SV40 specific RNA representing a full but non-spliced early transcript. The infected cells were T-antigen negative by immunoprecipitation and immunofluorescence.

These findings suggest that at least one block to SV40 gene expression in F-9 cells is related to the inability to process the primary transcripts. We have used the method of Strickland and Mahdavi for differentiation of F-9 cells in vitro. The retinoic acid treated F-9 cells were shown to be differentiated by a change in cellular morphology, the loss of the undifferentiated (F-9) cell marker and the acquisition of a differentiated (H-2b) histocompatibility marker. After differentiation, F-9 cells were able to support the growth of Py virus as well as the abortive infection by SV40. The relationship between cell differentiation and gene expression as monitored by papovavirus infection, will be discussed.

317 TERATOCARCINOMAS: A MODEL FOR VIRUS CELL INTERACTION IN A DIFFERENTIATING CELL SYSTEM, John M. Lehman and Thomas D. Friedrich, Department of Pathology, University of Colorado School of Medicine, Denver, CO 80262

The murine teratocarcinoma, when placed in tissue culture, consists of two cell types: 1) the stem cell, embryonal carcinoma, and 2) numerous differentiated cells which arise from the stem cell (1). The stem cell has been shown, when compared to mouse fibroblasts and to the differentiated murine teratocarcinoma cells, to respond differently to the papovaviruses, Simian virus 40 and polyoma (2). Differentiated cells are transformed by SV_{40} and lysed by polyoma. However, EC cells do not express any viral function, T antigen, V antigen, or production of infectious virus. We determined that the block to viral infection was not at the adsorption, penetration or uncoating step (3). Nor was interferon responsible for the block since EC cells neither produced nor were protected by interferon while the differentiated cells both produced and were protected by interferon (4). When the state of SV_{40} DNA in the EC cell was analyzed at 11 days after infection, the viral DNA consisted solely of unintegrated full length genomes that were capable of producing V antigen when introduced into monkey kidney cells. By 5 weeks after infection, no integrated or free viral DNA was detected. When EC cells containing SV_{40} DNA were differentiated by the chemical inducer, dimethylacetamide, the differentiated cells retained SV_{40} DNA in the nonintegrated state. Although no T antigen was produced by these genomes in the differentiated cells, they were capable of producing V antigen when introduced into monkey kidney cells. These results suggest that EC cells inhibit the expression of viral DNA, in a manner not seen in infected differentiated cells.

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Defined Culture Media for Anchorage-Dependent Cells

318 GROWTH AND FUNCTION OF CULTURED RAT GRANULOSA CELLS IN SERUM-FREE MEDIUM. Joseph Orly, Gregory F. Erickson and Gordon Sato, University of California, San Diego, La Jolla, CA 92093.
Ovarian granulosa cell obtained from immature hypophysectomized estrogen-treated rats were cultured in serum-free medium supplemented with fibronectin, insulin, transferrin, and hydrocortisone (4F medium). The growth curve in 4F medium was similar to that obtained in serum-containing medium, however in both media the cell number never exceeded more than one cell doubling. In serum-free medium without the supplemented factors, 90-95% of the cells died within 5 days in culture. The added factors also controlled the hormonally induced steroidogenesis in serum-free medium: omission of the factors resulted in a 94% inhibtion of the FSH-induced progestins secretion per cell. The FSH-induced steroidogenic activity per cell was 25-fold higher in 4F medium than in serum-containing medium. However addition of the factors mixture to the serum-containing medium did not elevate the steroidogenic activity of the cells indicating that rather than missing a necessary component, serum probably exerted an inhibitory effect on the hormonally induced steroidogenic pathway. Cholera-toxin and dibutyryl-cyclic AMP both stimulated progestins production even in the presence of serum further suggested that serum might have interfered with FSH binding or perturbed the coupling between the hormone-receptor and adenylate cyclase. FSH induced aromatase enzyme activity in granulosa cells cultured in 4F medium but failed to do so in serum-containing medium. Addition of androgen to 4F medium elevated the FSH-induced steroidogenesis and prolonged incubation of the cells in the presence of FSH and androgen resulted in morphological lutenization of the cultured cells.

319 EFFECTS OF MSA ON MYOBLASTS AND MYOTUBES, J. R. Florini, G. F. Merrill, D. Z. Ewton, C. M. Stoscheck, and M. R. Benedict. Biology Dept., Syracuse Univ., Syracuse, NY.
The roles of growth hormone (GH) and somatomedins in controlling the growth of muscle have been examined in studies of effects of GH and Temin's Multiplication Stimulating Activity (MSA) on rat muscle cells in culture. We have previously reported that MSA (but not GH) stimulates cell proliferation and amino acid uptake; the latter effect is not decreased as myoblasts fuse to form postmitotic myotubes. Further study of the effects of MSA on muscle cells has indicated: (1) All observed effects of MSA are closely paralleled by those of purified somatomedins, (2) The stimulation of myoblast proliferation by MSA is correlated with an increase in ornithine decarboxylase levels, but exogenous polyamines do not stimulate proliferation, (3) Extended exposure of cells to MSA is required to induce myoblast proliferation, (4) MSA (but not GH) stimulates AIB uptake and creatine kinase activity in postmitotic myotubes, (5) The interaction of ^{125}I-MSA with myoblasts appears similar to that between other growth factors and cells, (6) Supplementation of MSA-containing medium with insulin, dexamethasone, and fetuin (but not fibronectin) gives rates of cell proliferation comparable to those in 10% serum, and (7) The effects of MSA in myoblasts are mimicked by insulin at very high levels (10^{-6} M), but MSA does not completely replace insulin in stimulating myoblast proliferation. We conclude that the somatomedin family of hormones plays a major role in mediating the effects of growth hormone on muscle growth. (Supported by grants from the National Institutes of Health and the Muscular Dystrophy Association.)

320 TRANSFORMING FACTORS FROM ANCHORAGE INDEPENDENT CELLS, Paul L. Kaplan and Brad Ozanne, University of Texas Health Science Center at Dallas, Dallas, Texas 75235
Murine Sarcoma Virus (MSV), Simian Virus 40 (SV40), and Polyoma Virus (Py) transformed cells and spontaneously transformed Rat-1 cells produce a variety of growth factors which confer anchorage independent growth upon normal cells. Transforming Factor (TF) from Kirsten transformed normal rat kidney (KNRK) cells is stable to heat and acid, but sensitive to reducing agents. TF induces normal cells to assume a transformed morphology, to grow in soft agar, to grow to high densities, to grow in low serum, to increase hexose uptake, and to lose actin cable organization. Cells transformed by a temperature sensitive mutant of Kirsten MSV only produce TF when grown at the permissive temperature for transformation. Revertants of Kirsten MSV transformed cells no longer produce TF.

Transforming activities can also be isolated from anchorage independent SV40 and Py transformed cells. However, anchorage dependent SV40 and Py transformed cells are rendered anchorage independent by adding TF or factor(s) from anchorage independent SV40 transformants.

A tumor cell line derived from spontaneously transformed Rat-1 cells also produces TF. Although this factor and KNRK TF share many biological and physical characteristics, we do not know if the two factors are identical.

Our data show that transformation of cells by at least three different mechanisms (RNA viruses, DNA viruses, and spontaneously) involves the production of peptide growth factors. These factors appear to be necessary to initiate and maintain anchorage independent growth. We note that anchorage independent growth is most closely associated with tumorigenicity.

321 PHOSPHOETHANOLAMINE AS GROWTH-PROMOTING MATERIAL TO RAT MAMMARY TUMOR CELLS, T. Kano-Sueoka, J.E. Errick, and D. Schindele, University of Colorado, Boulder, CO 80309
Phosphoethanolamine has growth-promoting activity to a rat mammary carcinoma cell line , 64-24, in culture, which was derived from a highly hormone-dependent mammary tumor. Crude bovine pituitary extract was previously shown to contain a growth-promoting material to 64-24 cells, and subsequently this material was identified as being phosphoethanolamine. Phosphoethanolamine shows a considerable amount of growth-promoting activity when added in a serum-supplemented medium (5% calf serum), as well as in a serum-free medium supplemented with a combination of hormones. Dose-response studies indicate that 1 nmol/ml in the culture medium is sufficient to give significant mitogenic activity. Incorporation of ^{3}H-thymidine into primary culture of mid-pregnant rat mammary epithelial cells also seems to be stimulated by phosphoethanolamine. However, under our experimental conditions, the stimulatory effect is much less than that on 64-24 cells. The cell lines so far tested for the effect of phosphoethanolamine other than 64-24 cells are rat hepatoma cells, rat fibroblast cells, rat neurotumor cells, mouse 3T3 cells, and a hormone-independent variant of 64-24 cells. None of these cells shows a clear response to phosphoethanolamine.

Lymphocyte Effector Molecules and Clonal Growth of Lymphoid Cells

322 GROWTH STIMULATION OF BOVINE ENDOTHELIAL CELLS BY VITAMIN A, George Melnykovych and Chittoor K. Ramachandran, Kansas City VA Medical Center, Kansas City, MO 64128
In spite of the well known effects of vitamin A on various surface characteristics of cultured cells, it has been generally assumed that cells in culture do not require lipid-soluble vitamins as growth factors. Consequently, most defined media formulated for specific cell lines do not contain any of the lipid soluble vitamins some of which would normally be included with the serum supplement, bound to its various components. The availability of a simple procedure to delipidize serum without loss of serum proteins (B. E. Cham and B. R. Knowles, J. Lipid Res. 6:176, 1976) made it possible to demonstrate growth stimulation by retinol in a culture of bovine endothelial cells. The effect was evident after 3-6 days of incubation in the presence of from 10^{-8} M to 10^{-5} M retinol. At higher concentrations, retinol was toxic to these cells. The growth promoting effect which resulted in a 2-10-fold increase in cell yield was not the result of improved cell attachment to the substratum and was evident only in delipidized serum-supplemented medium. Retinol, retinal, retinoic acid and retinol acetate were all growth promoting.

323 SOMATIC CELL GENETIC ANALYSIS OF MAMMALIAN RIBONUCLEOTIDE REDUCTASE, Buddy Ullman, Lorraine J. Gudas, and David W. Martin, Jr., University of California, San Francisco, San Francisco, CA 94143.
Deoxyadenosine and deoxyguanosine are specifically toxic to lymphoid cells. Other purine and pyrimidine nucleosides also have profound effects on the rate of cell growth in culture. Biochemical genetic studies utilizing a murine lymphoma (S49) in cell culture have implicated the role of ribonucleotide reductase, the sole enzyme in mammalian cells which synthesizes *de novo* the deoxynucleotide precursors required for DNA synthesis, as the primary target in deoxynucleoside lymphotoxicity. DeoxyATP and deoxyGTP, the toxic metabolites of deoxyadenosine and deoxyguanosine, respectively, feedback inhibit ribonucleotide reductase, and deplete intracellular deoxyCTP concentrations to levels inadequate for normal DNA synthesis and cell proliferation. We have isolated and characterized mutant S49 cells whose ribonucleotide reductase activities have been altered in one of several regulatory allosteric sites. Among these cell lines are mutants which are insensitive to feedback by deoxyATP; insensitive to feedback by deoxyGTP; insensitive to feedback by TTP; supersensitive to activation by ATP; or resistant to hydroxyurea and guanazole, synthetic inhibitors of ribonucleotide reductase. The cell lines whose ribonucleotide reductases are refractory to complete inhibition by deoxynucleoside triphosphate do not become depleted of dCTP and are resistant to the growth inhibitory effects after incubation of cells with the respective deoxynucleoside. The somatic cell genetic analysis of mutant ribonucleotide reductase activities has led to considerable insight into the nature of the molecular function of this enzyme, and into its critical role in the regulation of DNA synthesis and cell growth.

324 IN VITRO STUDIES ON THE REGULATION OF LYMPHOID GROWTH AND DIFFERENTIATION

John W. Schrader, Ian Clark-Lewis and Perry F. Bartlett
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Little is known about the humoral regulation of lymphopoiesis. The advent of in vitro tissue culture systems however, has permitted the study of factors required for lymphocyte growth and differentiation. Supernatants of cultures containing activated T cells are known to contain factors affecting a range of cells including pluripotential hemopoietic stem cells, mature T and B lymphocytes and what are probably prothymocytes. It is not clear that the T cell is the actual source of the activities, as there is evidence that other cell types must be present in the cultures to generate activities. However we have demonstrated that a cloned T cell hybridoma can respond to the T cell mitogen concanavalin-A with the production of regulatory factors. This occurs in the absence of accessory cells and indicates that T cells themselves can produce factors regulating hemopoietic stem and progenitor cells, and T and B lymphocytes. The different activities produced in cultures of activated T cells appear to reside in several discrete molecules which can be differentiated by a range of criteria. These results suggest that the T cell has an important role in the regulation of lymphocyte generation and differentiation. In a second series of experiments we have studied in vitro the role of the bone-marrow in lymphocyte production. We have observed that Thy 1 positive small lymphocytes are generated in the Dexter long-term bone marrow culture system under the appropriate conditions. Data will be presented on the origin and characteristics of these cells and factors regulating their levels.

325 MECHANISM OF T CELL ACTIVATION, Eva-Lotta Larsson, Department of Immunology, University of Umeå, Umeå, Sweden

The mechanism by which mitogenic lectins induce murine T cells to grow have been dissected. 1) Direct interaction of specific ligands, at optimal concentrations, with "initiator" sites on resting T cell membranes induces the expression of growth receptors. This process is rapid (detectable after 3 h) independent of accessory cells and of the activity of microfilaments, but dependent on active metabolism and inhibitable by cyclosporin A. This "first step" in the triggering process does not result in growth and consequently "first step" ligands are not necessarily mitogenic. 2) Ligand dependent interactions between Thy.1-positive I.A-negative cells and Ig-negative, Thy.1-negative, I.A-positive cells leads to the production by the latter of a product with a MW between 15,000-20,000 which, in conjunction with the ligand, induces the secretion, by Thy.1-positive, I.A-negative cells, of a second active product with a MW around 35,000 (TCGF). This "second step" is also energy dependent, is profoundly inhibited by corticoids and it takes considerably longer than the "first step". TCGF production can proceed in cell populations which did not undergo "step one"; on the other hand, TCGF-reactive blasts are not competent to produce it. 3) Direct interaction of active TCGF concentrations with Thy.1-positive cells, which have undergone "step 1" and consequently express TCGF-receptors, induces the target cells to enter the mitotic cycle and initiate growth. This mitogenic process is also independent of accessory cells and the further clonal expansion is limited only by the availability of TCGF.

326 BIOSYNTHESIS OF DIFFERENTIATION MARKERS IN SUBPOPULATIONS OF MOUSE THYMOCYTES.

Ellen Rothenberg, The Salk Institute, San Diego, CA 92138

The cells in the mouse thymus which show assayable, mature immune function comprise a small minority population. They are separable from the functionally incompetent majority population by their low buoyant density and steroid resistance, and by their failure to bind peanut agglutinin. The two populations are also distinguishable serologically by their surface displays of H-2, TL, and Thy-1 antigens, and by their intracellular levels of terminal transferase (TdT) activity. To determine the basis for these phenotypic differences, thymocytes were pulse-labeled with (^{35}S)methionine and then fractionated into several populations. Lysates from each of the cell populations were then analyzed for newly-synthesized differentiation antigens by immune precipitation with appropriate antisera.
The rates of synthesis of TL and TdT were highest in dense, peanut lectin-agglutinable, cortisone-sensitive cells, and low or undetectable in low-density and cortisone-resistant thymocytes, and in cells not agglutinable by peanut lectin. Conversely, the rates of synthesis of the H-2 glycoproteins and Qa-1 (another MHC-linked differentiation antigen) were highest in the low-density, cortisone-resistant, nonagglutinable cells. Thus the well-documented differences in steady-state expression of these products reflect similarly marked differences in their biosynthesis in thymic subpopulations. Changes in these specific rates of synthesis may therefore be traced as possible intermediate events during the maturation of thymocytes into competent T lymphocytes.

327 PURIFICATION AND CHARACTERIZATION OF HUMAN LYMPHOCYTE ACTIVATING FACTOR-INTERLEUKIN I (LAF-IL I), Lawrence B. Lachman and Richard S. Metzgar, Duke University Medical Center, Durham, NC 27710

The functions of macrophages in the immune response include presentation of antigens and secretion of lymphostimulatory factors. LAF-IL I is the most highly characterized macrophage derived factor. LAF-IL I is mitogenic for mouse thymocytes and increased the plaque-forming cell response of normal and nude mice spleen cells by affecting a small population of helper T-cells. LAF-IL I is released by human peripheral blood monocytes when cultured for 24 hr. in medium containing normal human serum (NHS) and lipopolysaccharide (LPS). The release of LAF-IL I by human monocytes is dependent upon the presence of both LPS and NHS. Most tissue culture medium contains sufficient quantities of endotoxin to stimulate maximum release of LAF-IL I from human monocytes. Many macrophage stimulants which are believed to increase LAF-IL I release from monocytes are contaminated with endotoxin and therefore cannot be evaluated for their effect upon LAF-IL I release. The effect of endotoxin can be blocked by polymyxin B, indicating that the lipid portion of endotoxin is responsible for activity. Human LAF-IL I has been highly purified by a procedure of hollow fiber diafiltration and isoelectric focusing (IEF). Recently, improved yields (>10%) of LAF-IL I activity have resulted by eliminating concentration procedures in which LAF-IL I was recovered in poor yield due to non-specific adsorption to ultrafiltration membranes. LAF-IL I activity is reduced by treatment with proteolytic enzymes and cyanogen bromide but is unaffected by reduction with 2-mercaptoethanol. (Supported by grant CA 08975 and AM 08054)

328 PURIFICATION AND CHARACTERIZATION OF INTERLEUKIN II-TCGF, Diane Y. Mochizuki and James Watson, Department of Microbiology, University of California, Irvine, CA 92717

Factors present in the culture supernates of Con A stimulated spleen cells possess a number of biological activities as determined in four assay systems. The factor(s) is capable of (i) stimulating the continuous proliferation of T cell lines in culture, (ii) potentiating mitogen responses of thymocytes, (iii) inducing AFC in nude T cell depleted cultures, and (iv) inducing cytotoxic T cells in either thymocyte or nude spleen cell cultures. The four biological assays, together with standard biochemical techniques have allowed the quantitation and purification of a single class of lymphokine designated T cell growth factor (TCGF). The TCGF biological activities copurify after ion-exchange chromatography, gel filtration, and preparative isoelectric focusing (IEF). ^{125}I labeled IEF purified material is heterogeneous in SDS polyacrylamide gels. Elution of IEF purified factor from polyacrylamide gels will result in TCGF purification to homogeneity and will permit the assignment of biological activities to a specific factor(s). Characterization of the molecular nature of TCGF will be discussed in terms of the sensitivities of the biological activities to various chemical and enzymatic treatments.

329 DERIVATION AND CHARACTERIZATION OF DRUG AND LECTIN RESISTANT MUTANTS FROM CYTOLYTIC T-CELL LINES, Oreste Acuto, Andreas Conzelmann, Marcel North, Jean-Pierre Mach, Judy Johnson and Markus Nabholz, Swiss Institute for Experimental Cancer Research and Ludwig Institute for Cancer Research, CH-1066 Epalinges, and Institut für Mikrobiologie, Universität München, D-8000 München.

From normal cytolytic T lymphocytes cloned cell lines can be established which maintain their capacity to recognize and lyse suitable target cells. Karyotype analysis of several independently derived lines revealed a very large number of chromosomal rearrangements in all of them. The frequency of inactive variants in such lines is sufficiently low to permit their use as a source material for a somatic cell genetic analysis of their functional phenotype. Mutagenesis with doses of ethyl methane sulfonate which increase the frequency of 6-thioguanine resistant variants approximately one thousand fold does not lead to a drastic increase in the occurrence of inactive clones. Several stable variants resistant to 6-thioguanine and ouabain have been selected ; all of these maintain their lytic capacity. In addition lines with an increased resistance to the lectin from Vicia Villosa, which specifically binds to glycoprotein characteristic for cytolytic T-lymphocytes (T145) have also been obtained and partially characterized. Of twelve hybrids between a C57Bl/6 derived cytolytic T-cell line and the AKR thymoma BW5147 which express the Thy-1 and H-2 alleles of both parents none showed any lytic activity or expressed the Ly-2 marker characteristic of the B6-cell line.

330 COMPARISON OF THE BIOLOGICAL EFFECTS OF MITOGENIC FACTORS PRODUCED BY HUMAN MONOCYTES AND LYMPHOCYTES, Joost J. Oppenheim and Bonnie J. Mathieson, NIH, Bethesda, Md. 20205
Human monocytes when stimulated by endotoxin or colony stimulating factor (CSF) produce a 15,000 MW factor called lymphocyte activating factor (LAF) which is also called interleukin 1 (IL-1) that has a variety of intriguing in vitro biological effects. IL-1 is directly mitogenic for peanut agglutinin negative (PNA-) murine thymocytes. In conjunction with the lectin phytohemagglutinin, it is mitogenic for PNA+ as well as PNA- murine thymocytes. IL-1 also promotes thymocyte differentiation; it increases the expression of Lyt-1 antigens on thymocytes. IL-1 is only weakly mitogenic for peripheral human T lymphocytes. IL-1 promotes immunoglobulin production by pokeweed mitogen (PWM) stimulated (monocyte depleted) human B lymphocytes.

Human lymphocytes when activated by Concanavalin-A or antigens also produce a mitogenic factor called interleukin -2 (IL-2) that exhibits some distinct biological properties. IL-2 is directly mitogenic for both PNA- and PNA+ thymocytes, as well as for human T and B lymphocytes. IL-2 also promotes immunoglobulin production by PWM stimulated human B cells. Finally, only the IL-2 can support the long-term growth of cytotoxic T lymphocyte lines. These growth factors serve as second signals for selected lymphocyte subpopulations and thus may regulate and amplify immunological reactions by increasing both the lymphocyte number and function.

331 CLONAL ANALYSIS OF CYTOLYTIC T LYMPHOCYTES (CTL) GENERATED IN LIMITING DILUTION MIXED LEUKOCYTE MICROCULTURES (MICRO MLC), Janet L. Maryanski, K. Theodor Brunner, Jean-Charles Cerottini, H. Robson MacDonald, Carl Taswell and Michael B. Widmer, Unit of Human Cancer Immunology, Lausanne Branch, Ludwig Institute for Cancer Research, and the Department of Immunology, Swiss Institute for Experimental Cancer Research, 1066 Epalinges-sur-Lausanne, Switzerland.

A sensitive system of limiting dilution micro MLC has been developed to analyze the progeny of individual CTL precursor cells (CTL-P). Optimal sensitivity of the system was dependent on the presence of supernatant harvested from secondary MLC. By applying Poisson statistics, minimal estimates of the frequencies of CTL-P in various lymphoid tissues and in different mouse strain combinations have been determined. The frequency of CTL-P could be increased 50-100 fold by priming spleen cells for 4-5 days in conventional MLC, at which time up to 25% of the cells could be identified operationally as CTL-P. Even higher frequencies of CTL-P (up to 50%) were obtained in large-sized cells separated from day 4 MLC cells by velocity sedimentation. Preliminary studies indicate that CTL derived from individual micro cultures retain their specificity profiles when recloned. Thus, the system should allow a rapid screening of the specificity and function of large numbers of individual T cell clones in both allogeneic and syngeneic systems.

332 HUMAN MONOCYTE-MACROPHAGE DIFFERENTIATION IN VITRO: LOSS OF RECEPTOR FOR A CHEMOTACTIC PEPTIDE. J. Brice Weinberg, Joseph Muscato, and James Niedel. VA Medical Center, Duke University Medical Center, and Wellcome Research Laboratories, Durham, NC 27705
Various structurally related synthetic peptides are chemotaxins for human neutrophils and monocytes. Radioiodinated N-formyl-nle-leu-phe-nle-tyr-lys (S.A. 800-2000 Ci/mmole) is biologically active and binds to freshly isolated, adherent blood monocytes. The binding is rapid, saturable, and specific with an apparent K_D of 3-4 nM. After 60 minutes at 23°C with a saturating amount of peptide (20 nM), the monocytes bind 8.1 fmoles/10^5 cells indicating approximately 50-60,000 binding sites/cell. When blood monocytes are cultured in medium with 10% unheated autologous serum over several days, they become more spread and vacuolated and gain responsiveness to lymphokines as measured by nonspecific killing of transformed target cells. Associated with this apparent in vitro change from monocytes into macrophages, there is a decrease in peptide binding from 50,000 sites/cell in monocytes on day 0 to 1000 sites/cell in macrophages on day 8 when assayed using 20 nM peptide. To determine if this in vitro differentiation is comparable to in vivo differentiation, peritoneal macrophages from normal women undergoing laparoscopy were examined for peptide binding. Peritoneal macrophages are derived from blood monocytes and are morphologically similar to blood monocytes that have been in culture for 7 days. When assayed using 2 nM peptide, blood monocytes bound 3.1 fmoles/10^5 cells and peritoneal macrophages bound 2.9 fmoles/10^5 cells; after 7 days in culture, the peptide binding by each kind of cells had decreased by over 80%. Thus, human peripheral blood monocytes and peritoneal macrophages display comparable receptors for the synthetic chemotactic peptide, and in vitro culture of these cells causes loss of the receptors.

333 REGULATION OF THE PRODUCTION AND RELEASE OF HUMAN T-CELL GROWTH FACTOR (TCGF), Francis W. Ruscetti and Robert Gallo, NCI, NIH, Bethesda, Maryland 20205
Using conditioned media (CM) from phytohemagglutinin (PHA)-stimulated peripheral blood lymphocytes (PBL) we observed long-term selective growth of T-cells from normal human donors. The optimal method for preparing highly active CM from single donor PBL involves the addition of mitomycin C-treated B-lymphoblastoid cell lines to the mixture of PBL and PHA. A number of different cell lines greatly augmented the production of TCGF in 12/12 cases. Preparation of plasma membranes from the Daudi cell line could replace the intact cells in the production of TCGF but those from the cell line, Molt 4, could not. Since the cell surface of Daudi possesses HLA-D antigens but not HLA-A, B, and C, and Molt 4 has HLA-A and B and not HLA-D, it is possible that the immune response gene products are important in the release of TCGF. Using this method for growth factor production, an analysis was made concerning the events necessary for lymphocyte activation and the requirements for production and release of TCGF. Removal of PHA 12 hrs. after incubation had no effect on lymphocyte transformation but decreased TCGF release by 90%. In addition, vinblastine and cytosine arabinoside inhibited DNA synthesis but had no effect on TCGF release. Little or no TCGF activity was present after cellular protein synthesis was inhibited by puromycin and cycloheximide. These results suggest that TCGF production requires: a) protein synthesis; b) long-term binding of the stimulating agent; and c) occurs in a non-dividing cell, probably terminally differentiated, without the need for cellular proliferation.

334 LYMPHOCYTE TRIGGERING IN MINERAL DEFICIENT CONDITIONS BY T CELL REPLACING FACTOR (TRF) Arthur Flynn and Belinda R. Yen, Cleveland Clinic Foundation, Cleveland, OH 44106
Previous investigations have shown the immune response to be inefficient in the presence of mineral deficiencies. Long term in vivo Zn deficiency, for example, results in helper T cell defects and decreased immunoglobulin production. For our study, cytotoxic T cells were generated from normal Balb/c mice (8-12 weeks old) in vitro. This permitted the study of mineral deficiency effects without involving other regulatory systems that confound in vivo. Cells were cultured in RPMI 1640 with 10% fetal bovine serum (heat inactivated) with 1) no further treatment, 2) chelated with a chelating resin for divalent cations and repleated with all but one cation (Cu, Mg or Zn), or 3) completely reconstituted media. Generation of cell mediated lympholysis (CML) was totally abrogated by Cu and Mg deficiencies, whereas a 50% reduction in CML was noted in Zn deficient medium. T cell replacing factor (TRF) and normal control supernatant fluids were added to the three mineral deficient and two control media to investigate their ability to reconstitute CML. Our data showed addition of TRF to Cu deficient media could restore the generation of cytotoxic cells, whereas addition of TRF to Mg and Zn deficient media allowed for only a partial recovery of cell lysis. Normal supernatant fluids were not effective in reconstituting CML. Repleated media supported generation of CML equal to that supported by normal RPMI. These results suggest that Cu, Mg and Zn are required for helper cell activity and Mg and Zn are involved in additional ascepts of CML.

335 T CELL-MACROPHAGE SYNERGY INVOLVED IN THE GENERATION OF ALLOREACTIVE MURINE CYTOTOXIC T LYMPHOCYTES, James H. Finke, Somesh D. Sharma and Jeffrey W. Scott, Cleveland Clinic Foundation, Cleveland, OH 44106.
Synergy between T cells and macrophages is required for the production or release of a helper factor involved in the generation of alloreactive cytotoxic T lymphocytes (CTL). Cultures of activated T cells, primed *in vivo* to *L. monocytogenes*, and macrophages from nonimmune mice contained helper activity capable of assisting splenic T cells to develop into CTL when stimulated with heat-treated allogeneic thymocytes. Supernatant fluid from either cell type cultured alone were unable to provide "help" to splenic T cell responders. The primary helper activity had an approximate molecular weight of 35,000 daltons. In some experiments, weak helper activity was associated with molecules having a weight of 16-18,000 daltons. Both fractions exhibiting helper activity also had thymocyte mitogenic activity.

Further experiments suggested that activated T cell products induce macrophages to produce a factor that can provide the "second" signal to pre-killer cells. Supernatant fluids from activated T cells could provide helper activity for unseparated but not for nylon wool nonadherent responder spleen cells. Culture fluids of macrophages from mice stimulated *in vivo* with *L. monocytogenes* could assist in the generation of CTL when splenic T cells freed of adherent cells were used as responders. Culture fluids of Listeria activated macrophages, but not of T cells contained thymocyte mitogenic activity suggesting that at least one of the helper signals for generating CTL is separable from the mitogenic component.

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336 INTERLEUKIN 2 REGULATION OF IMMUNE INTERFERON. William L. Farrar and John J. Farrar
National Institute of Dental Research. NIH, Bethesda, MD 20205
The initiation of alloantigen-specific cytotoxic T lymphocyte precursors is dependent upon the presence of both macrophages and helper T cells or regulatory molecules derived from these facilitative cells. Three biochemically distinct helper factors have been identified: Interleukin 1 (macrophage-derived); Interleukin 2 (T cell-dependent); and immune interferon. All three factors are found in supernatants of mixed lymphocyte cultures (MLC), however the removal of macrophages from these cultures completely ablates the production of these factors as well as the induction of cytotoxic T lymphocytes (CTL). The addition of Interleukin 2 to these macrophage-depleted MLC restores, in a dose dependent fashion, the ability of the responder T cells to: 1) produce immune interferon and 2) generate CTL. The production kinetics of immune interferon in response to Interleukin 2 correlates with the generation of CTL. The production of Interleukin 2-induced immune interferon as well as the generation of CTL requires responder T cells and alloantigen but is independent of the presence of responder macrophages or B cells. These results suggest that the killer cell helper activity of Interleukin 2 may be bi-modal. In addition to the proliferative effects of Interleukin 2 on CTL or their precursors, Interleukin 2 may be involved in the regulation of another killer cell helper factor, immune interferon.

337 LONG TERM MAINTENANCE OF CLONED HUMAN T CELLS, Jacquelyn A. Hank, Hiroo Inouye, Barbara J. Alter, and Fritz H. Bach, Immunobiology Research Center, University of Wisconsin, Madison, WI, 53706.

Human T cells sensitized to alloantigen were cloned in soft agarose and by limiting dilution. The primed cells when grown in T cell growth factor (TCGF) containing medium undergo up to 25 divisions yielding approximately 1 x 10^8 cells. Once the cloned cells have undergone approximately 25 divisions proliferation often declines and eventually death of the cell population occurs, even though the TCGF used is active in allowing other clones, those that have not been in TCGF for as long, to proliferate. The long-term proliferation of cloned T cells in TCGF was enhanced by the utilization of irradiated Epstein Barr virus transformed (LCL) cells as a feeder layer. Clones have been maintained in culture for 150 days and it is possible to obtain greater than 1 x 10^{12} cells from an individual clone. Clones that expressed a proliferative response to the original sensitizing cells have maintained antigen specific activity in the primed LD typing (PLT) assay. Different clones from a single PLT reagent show different patterns of reactive responses on a panel of restimulating cells. Cloned PLT reactive cells should allow definition of HLA-D encoded antigens with a degree of specificity previously unobtainable with the average PLT reagent.

Some clones that did not show any proliferative response to any restimulating cells were cytotoxically active against the original sensitizing cell, but not to targets autologous with the responding cells.

338 LAF PRODUCTION BY HODGKIN'S DISEASE CELLS, Richard J. Ford and Abby L. Maizel, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030
Primary explants of lymphoma cells from patients with nodular sclerosing Hodgkin's (NHSD) were grown in short term (7-30 days) tissue culture. The Hodgkin's disease cells (HDC) in these cultures consisted primarily of large adherent mononuclear cells including binucleate giant forms resembling Reed-Sternberg cells (RSC) and occasional smaller lymphoid cells. The number of the large mononuclear cells in the cultures increased with time and were uniformly nonspecific esterase positive. Unstimulated supernatants from these cultures taken at various time intervals showed lymphocyte activating factor (LAF) activity on both murine thymocytes and purified human peripheral blood T lymphocytes. Non-adherent tumor cells, consisting of small lymphoid cells, granulocytes and eosinophils, when cultured for equivalent periods under the same conditions showed no LAF activity, and may represent reactive cells. The ability of the HDC to make LAF without lectin or other stimulation is of particular interest in that most of the conventional cell sources of this factor, including a neoplastic macrophage cell line (P388D1), have required lectin stimulation for significant activity. These findings support the concept that HDC are derived from macrophages and that the neoplastic macrophages are capable of secreting a lymphoproliferative monokine characteristic of normal macrophages, which may explain several of the unique features of this neoplastic condition.
This work was supported by Grants CA 25411 (RJF) and CA 21927 (ALM).

339 PROTECTIVE EFFECTS OF INTERLEUKIN-1 AND INTERLEUKIN-2 ON STEROID-SENSITIVE T CELL FUNCTIONS, Sally S. Fairchild, Kenneth H. Grabstein, and Robert I. Mishell, University of California, Berkeley, CA 94720

Two mediators, Interleukin-1 and 2 (IL-1 and IL-2), have been reported to regulate immune responses in vitro. We have shown that factor(s) physicochemically indistinguishable from IL-1 protect helper but not suppressor T cells from the inhibitory effects of steroids. Gillis et al. recently reported that the steroid inhibition of mitogen responses and the generation of CTL was reversed by IL-2. To determine whether partially purified preparations of IL-1 and IL-2 selectively affect different immune functions, we directly compared their abilities to protect T helper activity and responses to alloantigens and mitogens from the suppressive effects of dexamethasone (DEX). T cells were pretreated with DEX and with or without IL-1 or IL-2 for 2 days, washed, and then titrated for helper activity with T-depleted spleen cells. IL-1 protected more than 50% of the T helper activity; IL-2 had no detectable effects. In contrast, IL-1 provided insignificant protection against DEX to T cells which proliferate in response to mitogens or alloantigens; IL-2 has been reported to protect these activities. These data indicate the existence of two complementary pathways of protection against physiological concentrations of steroids. Experiments examining the capacity of the two mediators to affect suppression by dexamethasone of other T cell and macrophage functions are being conducted and will be reported.

340 VISUALIZATION OF A NOVEL TYPE OF PLAQUE FORMING CELL USING AN ANTISERUM AGAINST ALLOGENEIC CULTURE SUPERNATANTS, John C. Cambier and Ronald B. Corley, Division of Immunology, Duke University and Veterans Administration Medical Centers.

An antiserum was raised against 80% saturated ammonium sulfate insoluble material from 48 hr allogeneic culture supernatants. After adsorption to remove antibodies specific for mouse immunoglobulin and albumin, this antiserum precipitated a single molecular weight species from allogeneic supernatant as determined by SDS PAGE. This antiserum blocks the T cell replacing activity of allogeneic culture supernatants in B cell responses to SRBC. When used as the developing antiserum in a protein A reverse plaque assay, plaque forming cells were visualized in high frequency (> 1% of nucleated cells) among bone marrow, spleen and fetal liver cells, while being infrequent among thymus and lymph node cells. Data concerning the antigenic and physico-chemical relationship between moieties responsible for plaque formation and T cell replacing activity will be presented.

341 IDENTIFICATION AND PHENOTYPIC CHARACTERIZATION OF T-CELL GROWTH FACTOR (TCGF) PRODUCING T-LYMPHOMA CELL LINES. Steven Gillis and Margrit Scheid. Sloan Kettering Institute, New York, NY 10021

Recent studies have confirmed that the biochemically isolable protein;TCGF, acts as the key replication inducing trigger which drives both antigen and mitogen-induced T-cell proliferation. In hopes of isolating a stable tumor cell line source for TCGF, fifteen murine T-cell leukemia and lymphoma cell lines were screened for both constitutive and lectin-induced TCGF production. Only two such cell lines (RBL-3I and LBRM-33) produced high concentrations of TCGF following mitogen stimulation. In fact, PHA-stimulated LBRM-33 cells produced 10-50 times the amount of TCGF normally generated by identical stimulation of murine splenocytes. Surface phenotypic characterization of LBRM-33 (as assayed by isotopic protein A binding in the presence of monospecific antisera) revealed TCGF producer cells to be 95% Thy-1+, Lyl+, Ly2+, Q_a 2.3+, Qa3+ and minimally QaT4+. All fifteen tumor isolates were capable of absorbing high concentrations of TCGF (10 units/ml) during 4 hour, 4° c absoprtion tests. Absorption capacity directly correlated with the expression of cell surface receptors capable of saturably binding physiologic concentrations of ^{125}I-TCGF (23,000 Ci/M).Furthermore, 80% of the murine leukemia cell line samples tested, bound TCGF with higher affinity (i.e. at a lower molar concentration) than binding exhibited by normal Con-A activated murine splenocytes. Therefore, not only do malignant TCGF producer cells exist, the overwhelming majority of leukemic T-cell samples tested, resembled activated lymphocytes in terms of their ability to bind purified TCGF. These results provide interesting evidence suggesting a possible relationship between TCGF and malignant T-cell proliferation.

342 REGULATION OF POLYCLONAL IMMUNOGLOBULIN SECRETION, Gayle D. Wetzel and John R. Kettman, University of Texas Health Science Center, Dallas, TX 75235
Using conditions where culture wells of Terasaki plates contained a single input B cell, we have examined the capacity of splenic B lymphocytes from normal, adult BDF_1 mice to proliferate and differentiate to polyclonal immunoglobulin secretion in response to stimulation by the combination of two B cell mitogens lipopolysaccharide and dextran sulfate. We have previously shown that about 80% of the input B cells can be stimulated to undergo clonal expansion and that several different proliferative response patterns can be observed. Heterogeneity in both the burst sizes of individual clones and in the morphologies of expanding clones was observed. We have developed a modified spot test,using staphylococcal protein A coupled to sheep red blood cells, enabling the examination of immunoglobulin secretion by single, isolated B lymphocyte clones in our microculture system. Only a fraction, 10% to 25%, of the B cell clones induced by the combination of polyclonal activators secreted detectable levels of immunoglobulin. However, almost every B cell clone was induced to immunoglobulin secretion when grown in wells which contained 5 to 50 irradiated, adherent macrophages. The secreted immunoglobulin was predominantly of the IgM class. It appears that two subpopulations of B lymphocytes responding to mitogenic stimulation in this system can be defined. A minor population secretes immunoglobulin independent of other cell types whereas the majority of splenic B lymphocytes require macrophages for the secretion of detectable amounts of immunoglobulin.

343 LYMPHOID DENDRITIC CELLS ARE POTENT STIMULATORS OF MURINE CYTOTOXIC T-LYMPHOCYTES, M. Röllinghoff, K. Pfizenmaier and H. Wagner, Institute of Medical Microbiology, 65 Mainz, FRG.
In the past few years Steinman and Cohn have identified dendritic cells (DC) as a new cell type in mouse lymphoid organs. In the studies to be reported we have tested DC in regard to their capacity to stimulate alloreactive and H-2 restricted cytotoxic T lymphocytes (CTL). We found that partially purified DC are very potent stimulators for the in vitro induction of CTL. As little as 1000 DC were able to stimulate significant CTL reactivity in 5×10^5 splenic responder cells, while 5×10^4DC induced maximal cytotoxicity. This dose-response assay was used to compare the potency of partially purified DC with that of other heterogeneous lymphoid cell populations. The potency of DC as CTL-stimulators was 10-20 greater than that of unfractionated spleen cells. Removal of splenic B cells or T cells by anti Ig or with Thy 1.2 serum did not decrease the CTL-inducing capacity. Cell populations enriched for macrophages but lacking DC, had very poor CTL-inducing ability. We therefore conclude that DC are very potent CTL stimulators, being at least 10 times more efficient than B cells, T cells and macrophages.

344 LONG TERM CULTURE OF THY-1 BEARING CELLS ; DERIVATION FROM CONTINOUS BONE MARROW CULTURES. Gérard G. TERTIAN, Yee Pang YUNG and Malcolm A.S. MOORE. Sloan Kettering Institute, NEW YORK, NY 10021

Long term cultures of T cells can be established from different organs in mice including spleen, thymus and bone marrow by using a conditioned medium of concanavalin A stimulated lymphocytes as a source of T cell growth factor. In addition it has been shown that such a conditioned medium is able to induce the appearance of Thy-1 antigen on precursors present in the spleen of nude mice and to maintain these Thy-1 positive cells in long term cultures. We have used this system to investigate the presence of T cell precursors in the long term murine bone marrow culture system described by Dexter et al. where the mature Thy-1 positive T cell are lacking and shown that such marrow cultures do contain T cell precursors that can be induced to express the Thy-1 antigen in the presence of T cell growth factor. Long term cultures of T cells have been established from continous bone marrow cultures.

345 CHARACTERIZATION OF CYTOTOXIC T LYMPHOCYTE LINES AGAINST MURINE PLASMA CELL TUMORS, Janis V. Giorgi and Noel L. Warner, University of New Mexico School of Medicine, Albuquerque, New Mexico 87131

Cytotoxic T lymphocyte lines (CTLLs) against murine plasma cell tumors have been generated by priming normal spleen cells in vitro with syngeneic plasma cell tumors. The CTL lines have been maintained for as long as one year in medium supplemented with T cell growth factor. Much of our work has focused on analyzing the cell surface phenotype of these cell lines using flow cytometry. In these experiments, we have tried to correlate cell surface markers, including Thy 1.2, Lyt-1 and Lyt-2, with functional activities, including cytotoxic activity in vitro using the ^{51}Cr release assay, and protective immunity in vivo using the Winn assay. We have also raised antisera in syngeneic mice which reacts with the CTL lines, and we are currently investigating the reactivity of this antisera against a variety of cultured cell lines. Our results indicate that CTLL populations have reactivity against several plasma cell tumor antigens, and some of these responses are H-2 restricted and some are not. Furthermore, CTLL populations can be cloned, and these clones reflect the heterogeneous nature of the cytotoxic T cell response to tumor antigens. Cell surface phenotype analysis indicates that CTL lines are strongly Thy 1.2 positive, but the Lyt phenotype is variable, and changes during prolonged culture in vitro. However, high cytotoxic activity in vitro is reflected by good protective immunity in Winn assays. Further cell sorting experiments are in progress to try to enrich for functional activity in these CTLL populations on the basis of cell surface phenotype.

346 HELPER T CELL REPLACING FACTOR (TRF) ACTIVITY IN A T-DEPENDENT POLYCLONAL ANTIBODY RESPONSE, Marilyn L. Thoman, Edward L. Morgan and William O. Weigle, Scripps Clinic and Research Foundation, La Jolla, CA 92037

A helper T cell replacing factor (TRF), produced by Concanavalin A stimulation of murine spleen cells, replaces the requirement for T cells in a Fc-fragment-induced polyclonal antibody response. Human IgG1 Fc fragments stimulate proliferation and polyclonal antibody synthesis in murine spleen cultures. The polyclonal, but not the proliferative response, is T cell dependent. Neither Fc nor TRF alone stimulate a polyclonal response in T-depleted cultures, but together induce a response equal to, or greater than that generated by Fc in normal cultures. Macrophages are not required for delivery of the TRF signal. This suggests that TRF may act directly on the responding cells in this system. Fc and TRF appear to deliver independent, sequential signals to the responding cell. Fc fragments can be added up to 24 hours before TRF and still result in a maximal response, while the reverse does not allow a response. The material which restores the polyclonal response may be distinct from other activities found in Con A culture supernatants. When culture supernatants are purified by Sephadex G-100 chromatography and preparative isoelectric focusing, the majority of TRF activity which restores anti-erythrocyte antibody formation in T-depleted cultures and augments thymocyte mitogenesis resides in two peaks with pI values of 4.1 and 4.8. In this same preparation, the polyclonal restoring-TRF is found in one peak at pH 4.4. (Supported in part by USPHS grant AI07007 and USPHS training grant GM07437)

347 GROWTH OF T LYMPHOCYTE PROGENITOR COLONIES IN VITRO, J. John Cohen and Bonita R. Acuff, University of Colorado Medical School, Denver, CO 80262.

Progenitors of thymus-processed lymphocytes (pre-T cells) have been grown from mouse bone marrow in vitro. The necessary conditions include horse or human serum and spleen cell conditioned medium. Methyl cellulose is used to immobilize cells but is not essential. The cells which give rise to colonies are mostly cycling when the marrow is removed. They divide about every 10 h in culture. They are Thy-1 negative, but the colonies derived from them become Thy-1 positive and peanut agglutinin positive with time. According to our model of the control of pre-T cell proliferation (P.N.A.S., December 1979), the growth of these colonies should be dependent upon a thymic hormone. Preliminary experiments indicate that this is the case, offering the possibility of isolating, identifying and characterizing the thymic hormone. Furthermore, individual lines of pre-T cells can be established (R. Chervenak, personal communication) and immunological properties of these cells studied.

348 MOLECULAR CHARACTERIZATION OF THE LYMPHOCYTE STIMULATING FACTORS FROM HUMAN MONOCYTES. David D. Wood, Merck Institute, Rahway, N.J. 07065

When stimulated with endotonin, cultured human monocytes release factors which enhance the division of T cells and increase the number of antibody producing B cells. Chromatography of these supernatants on Sephadex G75 yields three major peaks of activity (JI, 123:2400, 1979). The strongest thymocyte mitogen elutes at 60,000 daltons. A factor which enhances the mitogenic response of thymocytes to PHA and the antibody response of splenocytes to heterologous erythrocytes elutes at 35,000 daltons. At 15,000 daltons, all three of these activities are found coeluting with the ability to enhance antibody production by T cell depleted spenocytes. Upon isoelectric focusing of the 15,000 dalton material, all the activities can be found at pI 6.5 with a substantial peak of activity also found occasionally at pI 4.5. The pI 6.5 15,000 elution material (termed interleukin -1) can be bound and eluted from an affinity column of rabbit anti-human endogenous pyrogen (prepared by Dr. Charles Dinarello). Using this affinity gel, the immunological relation of the other factors to interleukin -1 is under investigation.

349 AMPLIFICATION OF THE PROLIFERATIVE AND CYTOTOXIC RESPONSES TO ALLOANTIGEN AND TUMOR ANTIGEN BY A FACTOR PRESENT IN AN EXTRACT OF NORMAL SYNGENEIC THYMIC LYMPHOID CELLS. Peter W. Wright and Stephen M. Loop, Fred Hutchinson Cancer Research Center, Seattle, WA 98104

Synergy between subsets of thymic-derived (T) lymphocytes has been described for both the proliferative and cytotoxic responses to antigens. Previous studies from our laboratory have demonstrated that thymus cells from normal W/Fu rats were able to amplify the response of syngeneic peripheral T-cells to both alloantigens and tumor antigens in vitro. In the present series of experiments, a factor extracted from W/Fu thymus has been shown to retain amplifier activity comparable to that of intact thymus cells. Several features regarding this thymus factor have been established: (1) it is derived from thymic lymphoid cells (thymocytes) rather than thymic reticuloepithelial elements; (2) it acts on already differentiated T cells found in lymph node, spleen, and peripheral blood, rather than non-differentiated T-cell precursors. This latter observation is supported by studies using the FACS which have demonstrated that this factor acts primarily on a subpopulation of peripheral T-cells which express an antigen detected by the xenogeneic monoclonal antibody W3/25; (3) it is recovered with a 50-60,000 molecular weight fraction by Sephadex G-100 chromatography; and (4) it has no direct mitogenic activity, but its effects are observed only in the presence of an antigen or mitogen. Therefore, our thymus factor appears by virtue of its source, target cell, and physical properties, to be distinct from previously described thymus factors.

350 CLONES OF HELPER T CELLS SPECIFIC FOR HETEROLOGOUS ERYTHROCYTES, Max H. Schreier, Basel Institute for Immunology, Basel, Switzerland

Pure populations of specific murine helper T cells were obtained by longterm culture of in vivo primed splenic T cells followed by single cell cloning. They were maintained in vitro for up to 20 months in serum-free medium supplemented with T cell growth factor. Clones of helper T cells elicited to either sheep or horse erythrocytes demonstrated specific helper function both in vivo and in vitro. Syngeneic nude mice injected with cells from either clone and with homologous antigen produced specific antibody whose titers were 10- to 100-fold higher than those of immunized normal mice. No antibody formation above background could be observed against an unrelated antigen, even when homologous and unrelated antigen were injected at the same time. The in vitro antibody response induced with such cloned helper T cells could be dissected into two steps. In step 1, soluble helper activity could be induced by coculturing cloned specific helper T cells, I-A compatible adherent cells and homologous antigen. Such specifically induced T cell help could be assayed in step 2 by culturing T depleted spleen cells and antigen in the supernatant fluid of step 1. The induction of helper activity in step 1 was restricted to the presence of cells which were compatible to the left of the I-B region. The specificity and mode of action of antigen-activated T cell help functional in step 2 will be considered.

351 CELLULAR AND MOLECULAR REQUIREMENTS FOR COSTIMULATOR (IL2) PRODUCTION AND ACTION.
V. Paetkau, J. Shaw, I.F.C. McKenzie, B. Caplan and G. Mills, University of Alberta, Edmonton, Canada, and University of Melbourne, Australia.

Generation of the lymphokine costimulator (IL2) requires T lymphocytes, adherent (A) cells (probably macrophages) and a mitogenic or antigenic stimulus. Using antisera, we have shown that the T cell required is Lyl^+, 2^-, 7^+, and that the Lyl and 7 markers are on the same required cell. The requirement for an A cell is met by adding the purified factor IL1 ("LAF"), generated from a macrophage-like cell line. IL2 replaces both cell types required for its generation during the CTL response in vitro. That is, normal LN cells depleted of A cells and T "helper" cells can be induced to high levels of CTL activity with IL2, added at 10^{-10}M or less. Strong tumor-specific CTL activity can be obtained by incubating spleen cells from mice carrying a fatal, syngeneic tumor with costimulator and antigen for 5 days. The response rivals the allogeneic CTL reaction, and generates "killer" cells useful for enhancing in vivo clearance and enhanced survival. This approach fully uncovers suppressed antitumor responses in tumor-bearing mice, and allows us to differentiate this state from immunological unreactivity.

352 AN MHC-LINKED CELL SURFACE MARKER FOR ERYTHROID DIFFERENTIATION, B. M. Longenecker, and T. R. Mosmann, University of Alberta, Edmonton, Canada
The chicken major histocompatibility complex (B locus) codes for cell surface antigens expressed on lymphocytes but not RBC's, RBC's but not lymphocytes and on both types of cells. CBA/J mice were immunized with B^2/B^2 chicken RBC's and hybridomas which secrete specific anti-B^2 RBC monoclonal antibodies were constructed. One of these hybridomas (CH-4) which was found to secrete IgM monoclonal antibodies of high RBC agglutination titre (> 10^6) was selected for further analysis. In the presence of guinea pig complement, CH-4 was found to lyse only B^2/B^2 RBC's but had no effect on the viability or immunological activity of lymphocytes. In order to determine whether the B^2 RBC MHC antigen is expressed on erythroid progenitors an assay for chicken erythroid differentiation was set up. Pretreatment of bone marrow cells from B^2/B^2 chickens with CH-4 plus complement markedly inhibited the development of erythroid colonies but had no effect on the development of granuloyte-macrophage colonies. This suggests that the B^2-RBC antigen is expressed on erythroid progenitor cells and is a good candidate for a specific MHC-linked marker for erythroid differentiation.

353 MAINTENANCE AND REGULATION OF LYMPHOID PROGENITORS IN LONG TERM CULTURES OF MOUSE BONE MARROW, Robert A. Phillips, Ontario Cancer Institute, Toronto, Canada M4X 1K9
Dexter has described an in vitro method for maintaining myeloid progenitors for long intervals of time. When freshly prepared bone marrow cells are added to a previously established monolayer of lipid-containing cells from bone marrow, the stem cells in the second inoculum proliferate and differentiate for approximately 20 weeks. Such cultures contain multipotent spleen colony-forming cells and various committed myeloid progenitors such as CFU-C and BFU-E. However, the cultures lack both B and T lymphocytes. We have attempted to characterize the lymphoid potential of cells in long-term cultures. When cultured cells from CBA·T6T6 mice are injected into irradiated CBA recipients, large numbers of B and T lymphocytes with the T6 chromosome can be found in the irradiated recipients. This observation indicates that cultured stem cells retain their potential to produce normal lymphocytes and that the absence of lymphocytes in vitro results from optimal culture conditions. Tests on the effects of horse serum and hydrocortisone on B and pre-B cells indicate that both substances inhibit these activities. More recently we have found that a putative T cell progenitor, CFU-T, is also maintained in long term cultures. To investigate the importance of the adherent layer in the maintenance of lymphoid progenitors, we established adherent layers from Sl/Sl^d mice; others have shown that Sl/Sl^d adherent layers do not support myeloid differentiation. Although the lymphoid system is normal in Sl/Sl^d mice, the adherent layer from Sl/Sl^d mice supports neither myeloid (CFU-C) nor lymphoid (CFU-T) progenitors.

354 MODULATION OF THE NUMBER AND PROLIFERATIVE STATUS OF HEMATOPOIETIC STEM CELLS (CFU_S) BY THYMUS. Cullan, G.M., Anderson, R.W., Crouse, D.A., and Sharp, J.G. Department of Anatomy, University of Nebraska Medical Center, Omaha, NE 68105.

There have been conflicting reports regarding the effects of neonatal thymectomy (NTx) on the hematopoietic stem cell compartment (Zipori and Trainin, Exp. Hemat. 3:389, 1975; Stutman, Ann N.Y. Acad. Sci, 249:89, 1975). It also has been reported that stem cell (CFU_S) levels in NTx mice could be restored to normal levels by grafting intact thymus. This investigation was designed to evaluate the relative roles of cellular interactions and circulating thymic humoral factors (TF) in hematopoiesis. NTx BDF_1 mice were treated at 30, 60 or 90 days of age with a newborn thymus graft beneath the kidney capsule or with intraperitoneal transplantation of thymus in a cell-impermeable diffusion chamber. Other groups of 90 day old NTx mice were treated by administration of TF (thymopoietin, thymosin). The effects of the various treatments on hematopoiesis were assessed by determination of femoral cellularity, CFU_S content and hydroxyurea sensitivity. Since it has been postulated that TF may act by elevating intra-cellular cyclic AMP levels, another group of NTx mice was given isoproterenol. Results of this study demonstrated that CFU_S levels and bone marrow cellularity are significantly reduced following NTx. Hydroxyurea sensitivity of CFU_S from control and experimental mice did not demonstrate any significant alterations in the number of cycling CFU_S. Levels of CFU_S and bone marrow cellularity were restored following thymus grafting; treatment with TF or isoproternol. These results suggest that the mode of action of thymus and TF in this system may involve an increase of intra-cellular cyclic AMP levels. (Supported by NIH Grants AI 15819 and CA 18548.) [Thymopoietin & Thymosin kindly provided by G. Goldstein & A. Goldstein respectively.]

Culture of Hematopoietic Cells

355 ENRICHMENT FOR HEMOPOIETIC PROGENITOR CELLS USING FLUORESCENCE ACTIVATED CELL SORTING, Antony W. Burgess, Nicos A. Nicola, Suzanne M. Watt, Donald Metcalf and Francis L. Battye, The Walter and Eliza Hall Institute of Medical Research, AUSTRALIA, 3050.

The differential expression of surface carbohydrate moieties during granulocyte/macrophage (G/M) development has been used to purify normal murine hemopoietic progenitor cells. Pokeweed mitogen (PWM), Helix pomatia, soybean and peanut agglutinins showed a preferential quantitative binding to colony-forming cells (CFC). It was possible, using FITC labelled-PWM, to enrich up to 15-fold for the CFC's by selecting the high-ly fluorescent cells. Similarly, CFC were enriched up to 10-fold by sorting for cells with high intensity low angle (0°) scatter and low intensity high angle (90°) scatter. Combining these two light scatter parameters with the FITC-PWM binding distribution, it was possible to enrich for CFC's from bone marrow (35-fold), fetal peripheral blood (15-fold), fetal liver (20-fold), normal spleen (40-fold) and post-endotoxin spleen (40-fold). Ninety percent of the sorted cells were either blast cells or myelocytes and it was possible to unambiguously identify the morphology of CFC. The relative proportions of G/M, eosinophil, megakaryocyte, erythroid and multipotential CFC's in the highly enriched fractions, was identical to the distribution of the CFC in unfractionated cells. Two dimensional electrophoretic analysis, of the enriched progenitor cells in the presence and absence of purified G/M-colony stimulating factor, has been used to monitor early and late markers of myeloid development.

Tissue	Colonies & Clusters per 10^5 cells Unsorted	Sorted
Bone Marrow	400	20,000
Post-endotoxin Spleen	500	21,000
Fetal Liver	600	13,000
Fetal Peripheral Blood	3,000	40,000

356 SELF-RENEWAL CAPACITY AND OTHER PROPERTIES OF HUMAN T-LYMPHOCYTE COLONY FORMING UNIT IN CULTURE. A.M. Wu, Dept.Anatomy (Histology), University of Toronto, Ont. M5S 1A8.

To elucidate mechanisms underlying the differentiation and proloferation of T-lymphocyte colony forming unit (TL-CFU), some properties inherent to progenitor cells were examined using modified one step colony assay technique of Claësson et al. (Clin. Exp. Immun. 28, 526, 1977) carried out in 0.8% methylcellulose medium plus PHA. Our findings are: 1) The frequency of TL-CFU was one in 100 lymphocytes plated; 2)While E rosettes -forming T cells (E^+ cells) and their E^- progenitor T-cells from bone marrow could form T-cell colony in response to PHA stimulation, only E^+ T-cells from peripheral blood could; 3) All human TL-CFU in peripheral blood were in cell cycle with a ^{3}H-thymidine suicide rate of 40%. The generation time was 9 hours while the length of S phase was 3 hours; 4) TL-CFU are capable of renewing themselves upon repeat passages in methylcellulose medium. The burst size of each individual colony has a skew distribution with a modal value of 16 new CFU per CFU and maximum value of 80. In the presence of PHA and irradiated autologous leukocytes TL-CFU can be replated in methylcellulose medium for more than 4 times. In addition, the number of TL-CFU increased 10 to 70-fold during an 8 week period in a suspension culture containing T-lymphocyte growth stimulatory activity (TL-GSA) which are present in PHA-stimulated lymphocyte conditioned medium (Morgan et al. Science, 193, 1007, 1976 and Wu, Bull.du Cancer, 65, 421, 1978). Furthermore, although TL-GSA preparation enhanced neither size nor number of T cell colonies it enhanced the burst size of TL-CFU. This latter finding indicates, for the first time, a detection of some regulatory activity affecting the self-renewal capacity of T-lymphocyte progenitor cells.

357 HEMOPOIETINS FROM A HUMAN T-LYMPHOCYTE LINE. Aldons J. Lusis and David W. Golde, UCLA School of Medicine, Los Angeles, CA 90024

Our laboratory has derived a human T-lymphocyte cell line that constitutively produces several T-lymphocyte products, including colony-stimulating factor (CSF) and erythroid-potentiating activity (EPA). The cell line (Mo) was established from spleen cells from a patient with a T-cell variant of hairy-cell leukemia. The cells have been growing in culture for over two years and they maintain their T-lymphocyte properties. The CSF is continuously elaborated, and medium conditioned by the cells is 20% more potent than a human peripheral blood feeder layer in stimulating human granulocyte-monocyte colonies (CFU-C). Using serum-free conditioned medium, the Mo CSF has been purified to a specific activity of about 3 X 10^6 units/mg protein (1 unit = 1 colony/10^5 light density human marrow cells). Physically, the factor is an acidic glycoprotein of about 34,000 daltons molecular weight. The Mo cell line also produces a material that potentiates human erythroid colony formation in vitro. The EPA is easily distinguishable from CSF on the basis of differential heat stability. The Mo EPA has a molecular weight of approximately 45,000 daltons, is sensitive to proteases, binds to Con-A Sepharose and anion exchange resins, and has a pI of 3.5 - 4.5. It may be analogous to the "burst-promoting activity" present in mitogen-stimulated mouse spleen cell-conditioned medium. The Mo cell line should be an important source for the purification of human T-cell-derived humoral factors involved in the regulation of hematopoiesis.

358 RELEASE OF ERYTHROPOIETIN(Ep) FROM MACROPHAGES BY TREATMENT WITH SILICA I.Rich,V.Anselstetter,W.Heit & B.Kubanek.University of Ulm.W.Germany.

Mouse fetal liver and adult bone marrow and spleen suspensions were preincubated for 30 min with 10^{-4}gm/ml silica and the supernatant was added to 12 day fetal liver CFU-E target cells in the absence of exogenous Ep. A significant increase in colony counts above that which was obtained for controls and which in turn was higher than that found for spontaneous colony growth was seen. Silica-treated cells from the lung, liver and kidney also released the erythropoietic stimulating factor(ESF). Hypertransfusion decreases the ESF activity while bleeding increases the activity found in the supernatant from silica-treated bone marrow and spleen cells.In addition, when mice are injected with Actinomycin D and killed at various times thereafter,the ESF activity of the supernatant decreases between 30 and 60 min and cannot be detected at 4 hr. Bilateral nephrectomy plus hypoxia results in a greater increase in ESF activity released from bone marrow and splenic macrophages treated with silica than bilateral nephrectomy alone. Stimulation of erythropoiesis in polycythemic mice by a concentrated silica-treated spleen supernatant indicates that the ESF released from macrophages is active in vivo and similar if not identical to Ep. These results indicate that macrophages produce Ep a finding that has several important implications since it provides an explanation for extrarenal Ep production as well as a favorable microenvironment in the spleen for erythropoiesis. The effect of macrophages in vitro cannot be overlooked.

359 ERYTHROID PROGENITOR CELLS FORMING CLUSTERS IN VITRO: CHARACTERIZATION AND STATE OF DIFFERENTIATION, Francis C. Monette and P. Lynn Ouellette, Boston University, Boston, MA 02215

A number of early events in mammalian erythropoiesis have been recently characterized with the aid of in vitro clonal cell assays. However, the criteria for evaluating late erythroid progenitors (CFU-e) involves an arbitrary definition of eight or more cells. Since this may not allow the full magnitude of the in vitro erythropoietic response to be examined, this study was set-up to evaluate the characteristics of erythroid cluster-forming cells (defined as two to seven-celled clusters) in the microplasma clot culture system. Erythroid clusters and CFU-e were compared with regard to erythropoietin sensitivity, cell-cycle activity, response to hypertransfusion-induced plethora, average cell size, temporal kinetics of in vitro formation, and relative frequency in murine bone marrow. The results indicate that erythroid cluster-forming cells represent a stage of differentiation which is further along the erythroid pathway than CFU-e. Of particular interest was the relatively high sensitivity to erythropoietin which these cells demonstrated [linearity was observed over a dose range of 0.5 to 6.0 milliunits per culture] which suggested that these cells may provide the basis for a simple, rapid (24 hour) in vitro assay for the hormone at the milliunit level.
(Supported by PHS grants AM17735 and 00200.)

360 HEMOPOIETIC COLONIES ON THE CHORIOALLANTOIC MEMBRANE OF THE CHICK EMBRYO: INDUCTION BY EMBRYONIC, ADHERENT, NON-HEMOPOIETIC SPLEEN CELLS, Gordon Keller, Michael Longenecker, and Erwin Diener, Department of Immunology and MRC Group on Immunoregulation University of Alberta, Edmonton, Alberta, Canada, T6G 2H7

Granulocytic and erythrocytic colonies developed on the chick embryo chorioallantoic membrane (CAM) following the inoculation of chick embryo spleen cells. Dose response and kinetic experiments showed that the colonies are derived from cell aggregates present in the inoculum. Dissociation and reaggregation kinetics of the CAM colony-forming cells (CAM-CFC) indicate that the cells must be present as aggregates in order to form colonies. Results from the morphological and cell marker studies suggest that the colony forming aggregates (CAM-CFA) attract and support the differentiation of primitive host hemopoietic cells. The physical characteristics of the CAM-CFC, which are different from those of the hemopoietic progenitor cells, indicate they they represent a stromal cell population of the chick embryo spleen. Further evidence supporting this notion was provided by the radiation studies which showed that the colony-forming ability of the CAM-CFC is relatively radioresistant. The above characteristics of the CAM-CFC suggest that they represent the hemopoietic microenvironment of the chick embryo spleen.

361 ERYTHROPOIETIC ENHANCING ACTIVITY (EEA) DERIVED FROM A HUMAN CELL LINE: SIMILARITY TO COLONY STIMULATING ACTIVITY (CSA), C. N. Abboud, J. F. DiPersio, J. K. Brennan and M. A. Lichtman, University of Rochester School of Medicine, Rochester, New York 14642
Medium conditioned by the monocyte-like cell line, GCT, contains colony stimulating activity (CSA), a mediator of *in vitro* granulopoiesis. Also, the conditioned medium (CM) contains erythroid enhancing activity (EEA) which can be demonstrated in a system utilizing either nonadherent marrow or blood mononuclear cells, erythropoietin 1-2 units/ml, and 20% (v/v) fetal calf serum. Under these conditions, GCT CM enhances the growth of CFU-E and BFU-E. Attempts were made to determine whether EEA was different from CSA. Serum-free GCT cell CM was fractionated on Sephacryl S200 and Ultrogel AcA54. EEA and CSA cochromatographed with apparent molecular weights of ∿40,000 on Sephacryl and ∿30,000 daltons on Ultrogel. Fractionation on DEAE Sephacel led to an apparent separation of CSA from EEA; however, when diluted, the fractions containing CSA had EEA. Undiluted fractions contained potent CSA which inhibited erythropoiesis; however, dilution of these fractions resulted in marked EEA. Diluted crude GCT CM and DEAE Sephacel fractions enriched in EEA were capable also of sustaining BFU-E in liquid culture and mediating erythropoietin-independent colony growth. CSA could not be unequivocally separated from EEA on concanavalin A-Sepharose since the diluted void volume containing CSA also had EEA. EEA was present in CM boiled for sixty minutes, whereas CSA was markedly reduced but not abolished. Therefore, CSA and EEA are closely related activities, which stimulate granulopoiesis or erythropoiesis as a function of their concentration in culture.

362 ERYTHROID BURST PROMOTING ACTIVITY FROM HUMAN URINE, Peter P. Dukes, Andrew Ma and Dina Meytes, Depts. of Biochem. and Peds., USC School of Med. and Childrens Hospital of Los Angeles, CA 90054
Urinary proteins from patients with acquired aplastic anemia, Fanconi's anemia and iron deficiency anemia and from normal individuals were chromatographed on QAE-Sephadex A-50. Fractions containing a burst promoting activity (code named RP) were isolated. Addition of RP to mouse bone marrow cells cultured in a methylcellulose system to which no erythropoietin (EPO) was added, except for the trace amounts present in the fetal calf serum component of the medium, caused a dose dependent stimulation of erythroid burst colonies. Utilizing this assay up to 20 times higher RP levels were detected in urines from anemic patients than the RP levels found in the urine of normal individuals. The ratio of RP activity to EPO activity varied markedly from urine to urine.

The subcolonies of the bursts induced by RP were all made up of large, lightly hemoglobinized cells and were larger and more numerous per burst than those found when EPO was used as the inducer. One-day preincubation of marrow cells with RP, followed by washing, increased their subsequent burst response to EPO by 149 ±25% (four experiments) over that of controls preincubated in the absence of RP. Preincubation with EPO had no effect. Aplastic anemia RP preparations when added to mouse marrow cell cultures did not support CFU-E derived colony formation, nor did they stimulate granulocyte-macrophage colonies. RP stimulated human peripheral blood erythroid burst formation.

It seems that RP functions as a regulator of erythropoiesis modulating the number of early progenitor cells.

363 IS ERYTHROPOIETIN THE ONLY FACTOR WHICH REGULATES LATE ERYTHROID DIFFERENTIATION?
Barbara Fagg, ISREC, CH-1066 Epalinges s./Lausanne, Switzerland.
The maturation of late erythroid precursor cells (CFU-E) to give colonies of approximately 60 terminally differentiated cells after 2 days in vitro has previously been regarded as strictly erythropoietin (Epo)-dependent. However, evidence is presented here which indicates that another factor(s) contained in spleen cell-conditioned medium (CM) is also able to support the maturation of CFU-E from mouse bone marrow and fetal liver. In the absence of exogenous Epo, CM-treated bone marrow developed benzidine-positive colonies after 2 days in culture in proportion to the number of cells plated. The number of colonies formed was linearly related to the amount of CM added to cultures at low doses, reached a maximum value at approximately 4% (v/v) CM and declined at higher doses. The maximum response obtained with CM was 40-90% (depending on the batch) of the maximum Epo-induced value. This pattern was not altered when CM was dialysed and similar results were obtained under serum-free culture conditions. The factor in CM was stable to heating at 56°C for 30 minutes but progressively lost activity after more than 5 minutes at 100°C. Unlike Epo, most of this activity was retained on ConA sepharose columns and could be eluted with α-methyl mannoside, along with granulocyte/macrophage colony stimulating activity. Chromatography on Sephadex G100 indicated that the apparent molecular weight of the factor(s) is 25,000-40,000, similar to granulocyte/macrophage colony stimulating activity. These experiments suggest that the factor(s) from CM which stimulates CFU-E maturation is probably not Epo, and work is in progress to characterise this factor(s) in more detail.

364 BONE MARROW ORGANIZATION IN VITRO
Ursula Reincke and Philip Hsieh, Department of Radiation Therapy and Sidney Farber Cancer Institute, Harvard Medical School, 50 Binney Street, Boston, MA 02115
Long term cultures of mouse bone marrow can be maintained on glass coverslips in a Dexter-type system which involves a single cell inoculum and weekly feedings of 36% conditioned and 64% fresh medium without cell replacement. The complex system of several collaborating cell types is remarkably similar to normal, uncultured bone marrow. Established cultures show a characteristic architecture where reticulum cells and histiocytes form a partially open, partially confluent meshwork. Blood forming cells are either granulopoietic or blast-like and arranged in colonies or bursts on confluent stroma areas. The organization into sparse and dense areas and functionally supportive role of the stroma led us to study the marrow cultures in terms of large, external, transformation-sensitive (LETS) protein. LETS protein or fibronectin is the only cell surface component so far shown to correlate with abnormal morphologic characteristics of transformed cells. Its role in normal cell ecology is still unclear (Chen et al, Ann NY Acad Sci 312, 1978, 366). We examined live cultures in phase contrast and dark field, demonstrated the fibronectin by indirect immunofluorescence technique, and compared the observed structures with those on fixed and May-Gruenwald Giemsa stained coverslips. We saw a characteristic and transient pattern of events during the period of initial culture establishment where highly active, RNA-rich reticulum cells appeared to congregate to form numerous rug-like sheets. A similar pattern could be obtained in older cultures upon infliction of a mechanical scrape wound.

365 EXISTENCE OF TWO LEVELS OF NEGATIVE FEEDBACK INHIBITION OF MEGAKARYOPOIESIS
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It has been proposed that megakaryopoiesis is separately controlled at two stages of development; at the clonable progenitor cell and at the level of the maturing megakaryocyte. Both compartments were analyzed in animals in which megakaryopoiesis and platelet shedding are perturbed by transfusion of platelet concentrates. Animals (rats) receiving platelet concentrates developed a thrombocytosis of approximately three times baseline values. No changes in WBC and hematocrit levels were seen in any group including the controls (saline and platelet-free plasma injections). A humoral inhibitor in the sera of the thrombocytotic animals significantly reduced platelet counts and platelet production (^{75}SeM incorporation) in recipient mice. Although this sera had a marked effect on platelet production, varying amounts of this sera caused no alteration of detectable CFUmk levels. The effects of thrombocytosis on the differentiated cell compartment were to suppress all levels of megakaryopoiesis from the earliest cells (acetylcholinesterase-positive cells, SACHE) to platelets. The sequential effects were: reduction in SACHE, reduction in megakaryocytes, reduction of platelet numbers and reduction of platelet count. This suppression began on day three post-transfusion and reached maximal inhibition on day seven. Values returned to normal by day nine. This study indicates that two levels of regulation may exist in the negative feedback regulation of megakaryopoiesis.

366 REGULATION OF HUMAN MYELOID STEM CELL PROLIFERATION BY PROSTAGLANDIN E AND LACTOFERRIN.

Louis M. Pelus, Hal E. Broxmeyer and Malcolm A.S. Moore. Sloan Kettering Institute for Cancer Research, New York, N.Y. 10021.

Studies on the regulation of human myeloid stem cell proliferation indicate that progenitor cell populations committed to monocytoid differentiation are preferentially inhibited by Prostaglandin E (PGE). Lactoferrin (LF), when added to cultures of peripheral blood or bone marrow monocytes or macrophages results in the inhibition of colony stimulating factors (CSFs) necessary for clonal monocyte/macrophage, neutrophil and eosinophil expansion.
The addition of PGE to day 7 CFU-c cultures results in the dose dependent inhibition of total colony and cluster formation. Morphological analysis of proliferating clones revealed that the effect of PGE on total clone formation resulted from the selective inhibition of monocytoid colony and cluster formation. Mixed monocytoid/neutrophil colony formation was markedly less sensitive and neutrophil and eosinophil colony formation essentially insensitive to the inhibitory effects of PGE. The inhibition of colony formation by PGE extended equally well to day 14 CFU-c.
The addition of iron saturated LF to liquid cultures of human peripheral blood or bone marrow monocytes/ macrophages results in the inhibition of CSFs necessary for day 7 and day 14 monocytoid and/or neutrophil and eosinophil colony and cluster formation. Likewise, the inclusion of LF into mononuclear cell feeder layers or addition to agar cultures proliferating spontaneously, in response to endogenously produced CSFs, results in the equivalent inhibition of all morphologically identifiable myeloid clones.
These results implicate PGE and LF in the dualistic regulation of myeloid stem cells.

367 CHARACTERIZATION OF CLONED LYMPHOHEMATOPOIETIC STROMAL CELLS.

Anderson, R.W. and Sharp, J.G., Dept. of Anatomy, Univ. of Nebr. Med. Center, Omaha, NE 68105.

Considerable evidence indicates that the proliferation and differentiation of both hematopoietic and immunologically active cells is influenced differentially by the microenvironmental mileau provided these cells in various lymphohematopoietic tissues. Several investigators have suggested that the adherent 'stromal' cell populations which grow as colonies in cultures of these tissues include the cells involved in such regulatory processes. Grossly, the colonies observed by several investigators are similar morphologically and the cells giving rise to them have been variously termed 1) fibroblast colony forming cells (FCFC), 2) plaque forming units-culture (PFU-C), 3) macrophage colonies and 4) marrow stromal cells. These cells reportedly are able to re-establish their parent microenvironment when transplanted in an allogeneic system. However, this observation has been difficult to duplicate in a syngeneic mouse system. We have cloned cells from several colonies obtained from cultures of lymphohematopoietic cells. These cloned cells have been characterized by their growth in culture and using morphological, histochemical and electron microscopic techniques. The results demonstrated that although the initial stromal colonies appeared to be identical, the constituent clonable cell type varied considerably. Some colonies have been shown to be composed primarily of macrophages, while others appear to contain predominantly fibroblasts. One further cell type which established such colonies has not yet been satisfactorily identified. These results demonstrate the need for caution in the analysis of experiments in which uncharacterized stromal cell colonies are transplanted in an effort to evaluate the origins and functions of lymphohematopoietic stroma. Supported by Grants CA18548 and AM26636.

368 TWO-FACTOR REQUIREMENT FOR MEGAKARYOCYTE COLONY FORMATION,

Neil T. Williams, Richard R. Eger and Heather M. Jackson, Sloan-Kettering Institute for Cancer Research, 145 Boston Post Road, Rye, NY 10580

Entities which permit cloning of megakaryocyte progenitor cells (CFU-MK) are collectively termed megakaryocyte colony-stimulating activity and are derived from erythropoietin preparations, as well as conditioned medium from mitogen-stimulated spleen cells, lung, and a myelomonocytic leukemic cell line (WEHI-3). Mature megakaryocytes may be obtained using those unfractionated culture supernatants. After fractionation of WEHI-3 cell-conditioned medium by gel filtration, CFU-MK do not clone unless mixing experiments are performed or assays are set up at high cell concentrations ($>10^5$ cells/ml). One factor in WEHI-3CM appears to be similar to MK-CSF. It is obligatory at all cell concentrations cultured. The second activity is obligatory only when less than 75,000 cells are cultured. At higher cell concentrations it appears to be endogenously produced in limiting amounts. A nonlinear increase in CFU-MK numbers is observed when increasing numbers of cells are plated. Addition of this second activity to the cell cultures optimized conditions allowing quantitative analysis of megakaryocyte progenitor cells. Exogenous sources are detected by enhancing the incidence of megakaryocyte colonies grown in the presence of the obligatory factor. The potentiating activity is also elaborated from bone, peritoneal exudate and lung. Each factor may have a separate role in megakaryocyte development.

Gene Organization and Reorganization

369 THE ARRANGEMENT OF HISTONE H3 IN CHROMATIN AS INVESTIGATED BY A FLUORESCENT PROBE,
H. Simpkins & D. Mooney, University of California, Irvine, Irvine, California 92717.
The use of fluorescent probe which specifically labels certain amino acid residues has been used in the past to monitor reassociated nucleosomes where histone H3 was labelled prior to reassociation of the nucleosomes with N-pyrene maleimide. The use of this probe to label intact chromatin has not been investigated or reported. We have used this probe to label rat liver and mouse thymocyte chromatin with two cysteine residues/H3 molecule. However, we found that chick erythrocyte chromatin with histone H3 which contains only one cysteine residue at position 110 did not label. The spectra of isolated H3 from rat liver and that from chromatin was identical; however, the kinetics of labelling were completely different as the isolated histone H3 labelled at a far greater rate than histone H3 existing within the intact nucleosome. We have used this probe to monitor the quenching of non-histone protein tryptophan fluorescence. The probe has been used to show structural changes in chromatin from regenerating rat liver vs. control sham operated animals. The fluorescence of the probe was greater although the kinetics of binding did not appear markedly different. The composition of the two chromatin preparations were similar and the fluorescence change decreased at zero at approximately 72 hours post-hepatectomy.

370 MOLECULAR BASIS FOR HISTONE GENE EXPRESSION,
Allan Lohe, Molecular Biology Institute, University of California, Los Angeles, California 90024
The bulk of the histone genes in sea urchins are organized in repeated copies, each copy containing the five histone genes. There are about 500 such copies repeated tandemly in S. purpuratus. Histone genes are activated immediately following fertilization, and molecular cloning has enabled the repeats (6.5 kb in length) to be characterized with respect to gene order, spacerlengths, and most of the nucleotide sequence.

A histone gene switch occurs at about 12 hours after fertilization so that mRNA synthesis from the predominant, "early"-type histone repeats ceases and is replaced by mRNAs from newly-activated "late" histone genes. Little is known about the structure of "late" histone genes and a lambda library containing partial EcoR1 fragments was screened for the "late" genes. No "late" genes were detected in this library, indicating that these genes may not contain an EcoR1 site and are absent from the library. We are currently constructing a more complete sea urchin library which should contain these histone genes.

Sequence comparisons of corresponding "early" and "late" gene regions will be important in understanding the evolution of these genes and the mechanism of gene activation during animal development.

371 *THE EPIGENETIC ADDRESS: A MODEL FOR CELLULAR DIFFERENTIATION DURING EMBRYOLOGICAL DEVELOPMENT,*
Wilfred D. Stein, Hebrew University, Jerusalem, ISRAEL
At each stage in development a cell is part of a lineage of cells, changing its epigenetic status in response to extracellular or intrinsic signals. These signals may be of only two classes, A or B. The current epigenetic status of a cell, its address, is a result of the particular set of signals it received during epigenesis. One cell type may be represented as ABBABAB, another ABABABA, etc. Two models by which such signals can be recorded in the chromatin will be presented. One, in which cell differentiation results from a differential transcribing of DNA, records the epigenetic address on the nucleosomes. The second, for total transcription followed by post-transcriptional RNA processing, postulates "address-RNA", an RNA species peculiar to each cell type, controlling the differential processing of messenger RNA. The gene for each luxury protein of every cell type contains both regulatory information (sequences of A,B instructions corresponding to the cell address at which the protein is required) and structural information. Thus, all luxury proteins which are expressed in a cell type with address ABBABAB will be coded for by base sequence ABBABABSSS where A,B... are regulatory and S,S... are structural sequences.

372 DNA-MEDIATED TRANSFER OF THE tk^+ GENE IN MOUSE L CELLS: EXPERIMENTAL AND GENETIC FACTORS AFFECTING COMPETENCE, Mark L. Pearson and Cheryl M. Corsaro, Frederick Cancer Research Center, Frederick, MD 21701
Somatic cells in culture can be transformed by unfractionated mammalian DNA precipitated with calcium phosphate. The efficiency of transformation is of the order of 10^{-6} under standard conditions - a value similar to the spontaneous mutation frequency for many markers of interest. For the method to be widely applicable to the analysis of gene structure and function, it would be useful to raise this efficiency. Using mouse LM tk^- cells and the thymidine kinase tk^+ gene as a test system, we have found that exposure of the recipient cells for longer times (24 vs 4 hr) and increasing the expression time (48 vs 24 hr) can result in a 20-fold increase in the frequency of transformant clones. Segregation analysis shows that transformants fall into two classes, stable and unstable, when grown in the absence of selective pressure, and the unstable class is more frequent (6/7 clones tested). After back selection to the Tk^- phenotype, transformed recipients appear to be more susceptible to retransformation by 3- to 7-fold. Therefore efficient DNA-mediated gene transfer seems to depend on genetic as well as environmental factors. We are currently testing the generality of these observations using other genetic markers and other recipient cell lines.

373 ISOLATION AND CHARACTERIZATION OF THE β^A, β^C, γ, AND EMBRYONIC GLOBIN GENES FROM THE GOAT, Joel R. Haynes, Kate Smith, Paul Rosteck, Jr., Eric A. Schon, Patricia M. Gallagher, Douglas J. Burks, and Jerry B. Lingrel, Department of Biological Chemistry, University of Cincinnati, College of Medicine, Cincinnati, Ohio 45267.

Bacteriophage containing globin DNA sequences were selected from a goat DNA recombinant library by hybridization to globin [^{32}p]cDNA. Eight clones were shown to carry β-like sequences and three were found to contain α-like sequences by hybridization to plasmids containing either mouse α- or β-globin cDNAs. Restriction endonuclease cleavage maps were determined for each of the β-like clones and selected fragments were isolated for DNA sequencing. Recombinants carrying genes for the goat β^A, β^C, and γ globins were identified by comparing the amino acids coded for by the cloned DNAs to those of the various goat globins. In addition, a clone was tentatively identified as containing an embryonic globin gene because the amino acid sequence it codes for more closely resembles that of the human embryonic globin than those of the goat β^A, β^C, or γ globins.

Detailed analysis of the clone containing the β^C gene revealed that a region of β-like sequences is located about 6 kilobases on the 5' side of the β^C gene. The amino acids coded for by this gene are only 50% homologous to those of the various goat globins and in addition, a translation termination codon is not present in the position expected for the known globins. Another presumptive globin gene is found about 5 kilobases in the 5' direction from this unidentified gene.

374 ELUCIDATION OF THE STRUCTURAL ORGANIZATION OF A CLONED SHEEP PRO α2 COLLAGEN GENE BY R LOOP MAPPING, Millie P. Schafer, Charles D. Boyd, Paul Tolstoshev and Ronald G. Crystal, NHLBI, Bethesda, MD 20205
Type 1 collagen, composed of two α1(I) and one α2 polypeptide chains, is the most abundant of the 5 known mammalian collagen types. To begin to elucidate the structural organization of these collagen genes, SpC3, a recombinant phage containing a 17 kb genetic insert representing approximately 60% of the sheep collagen pro α2 gene, was evaluated by electron microscopic R loop analysis. Only 3 kb, interspersed throughout the 17 kb fragment, coded for pro α2 collagen information. Thus, the DNA region required for coding the 3 kb of pro α2 collagen genetic information was 5.6 fold longer than the corresponding pro α2 mRNA coding sequences, a ratio similar to that reported for other large eukaroytic genes. A complex R loop pattern was determined containing 18 exons and 17 introns. Fourteen of the 18 exons were less than 150 bp in length. The largest exon, located at the extreme 3' end of the insert, contained approximately 800 bp. Restriction mapping and Southern blot analysis using a ^{32}P-labelled pro α2 cDNA probe suggested that this exon coded for information near the 3' end of pro α2 mRNA. The length distribution of the 17 introns ranged from 300 to 1600 bp occupying a total of 14 kb. If the remainder of the pro α2 collagen gene has a structural organization comparable to the 60% 3' portion that was analyzed, it is probable that the pro α2 collagen gene in its entirety is dispersed throughout a 30 kb DNA segment and contains 25-30 introns.

375 SOMATOSTATIN cDNA SEQUENCES IN ANGLERFISH, P.Hobart, R.Crawford, L.Shen, R. Pictet, W.Rutter, Dept. Biochem. & Biophys. Univ. Calif. San Francisco, Calif. 94143.

We have prepared a library of cDNA clones synthesized from mRNAs present in the Brockmann Bodies of Anglerfish (Lophius americanus). In contrast to the mammalian pancreas, the anglerfish Brockmann Body is a pure population of pancreatic endocrine cells with a high proportion of A (glucagon producing) and D (somatostatin producing) cells. Using Southern blots, cloned cDNA sequences representing frequent mRNAs in the poly A+ RNA fraction were selected and further subgrouped according to their ability to cross hybridize with each other. By sequence analysis we have determined the existence of two somatostatin ("-like") genes among these subgroups. Translation of the cDNAs indicates that one fish somatostatin has the same amino acid sequence as the mammalian hormone while the second cDNA encodes a peptide differing by two amino acids. However, both the somatostatin and the somatostatin-like peptide coding sequences are located at the COOH-terminus of a large precursor, are able to be cleaved from their respective precursors at a lys-arg amino acid sequence, and both precursors are encoded by mRNAs of similar size (575 $\pm$25 bases--Northern blot analysis). The cloned cDNA fragment encoding somatostatin is 560 bps in length. In phase with the translation reading frame of somatostatin is a methionine residue at bases 38-40 and is thought to start the precursor peptide. Thus the molecular weight of the precursor is approx. 13,300 dal. It is presumed that the somatostatin-like precursor is of a similar size. Based on a partial sequence of the somatotstatin-like cDNA, there is very little nucleic acid homology between the two cDNAs beyond the area coding for the hormones. The data suggests that, in fish, a family of somatostatin genes may be expressed in the endocrine pancreas.

376 ISOLATION OF FISH PROINSULIN cDNA. R. Crawford, L. Shen, P. Hobart, R. Pictet & W. Rutter. Department of Biochemistry & Biophysics, University of California, San Francisco, CA 94143.

We are interested in the evolution of the insulin gene, and are aiming to gain information by comparative analysis of its structure in various vertebrates. We have cloned an anglerfish insulin cDNA. In this species the endocrine pancreas is a separate organ, and this feature facilitates the isolation of pancreatic endocrine mRNA. A rat insulin cDNA probe did not hybridize with the fish cDNA clones. Thus we selected the clones for sequence on the basis of their frequency in the total population of recombinants, knowing the proportion of B cells in the tissue. Compared with rat and human insulin mRNA we observed

1. The anglerfish insulin mRNA is about 200 nucleotides longer,(by Northern analysis)
2. The 3' untranslated region is 219 nucleotides, compared with 53 in rat, but the AAUAAA sequence is located at about the same distance (15 nucleotides) from the polyA.
3. The fish C peptide is 40 amino acids in length, in contrast to the known mammalian C peptides, which are 35 amino acids long. In addition, the fish C peptide is connected to the B chain by only 1 arginine, suggesting that there is a different enzymatic activity for the conversion of fish proinsulin to insulin.

377 CONTEXTURAL GENOMIC RE-ARRANGEMENTS OF VARIANT ANTIGEN GENES IN TRYPANOSOMA BRUCEI Richard O. Williams, Kenneth B. Marcu*, John R. Young, Phelix A.O. Majiwa, Int'l Lab for Research on Animal Diseases, Box 30709, Nairobi,Kenya; *Dept. of Biochem., SUNY Stony Brook, N.Y.

The hemoparasitic protozoa belonging to the genus Trypanosoma cause extensive disease problems in both livestock and humans in Africa. These parasites are able to maintain a chronic pathologic infection by evading or limiting the immune capabilities of the host. It is believed that one of the mechanisms contributing to this chronic infection is the ability of the parasites to alter their membrane antigens. Populations of T. brucei brucei derived from cloned cells are capable of completely changing the antigenic structure of their membrane surface. The cell membrane of T. brucei contains a single predominant glycoprotein (MW 65,000) on its external surface which represents 95% of the total membrane protein. cDNAs have been prepared from mRNAs coding for two variant specific surface antigens and cloned in pBR 322. Southern blot hybridisation experiments have been performed between the cloned structural genes and genomic DNAs. There are DNA contextural rearrangements in the region of the variant antigen genes between populations of cells expressing different variant antigens (Williams, Young and Majiwa, Nature in press). Genomic libraries have been made in Lambda phage Charon 4A and have been screened with the cloned sequences. We will present results describing the arrangements of the genomic variant antigen genes in the expressed and the non-expressed state.

378 VARIATION OF IMMUNOGLOBULIN HEAVY CHAIN CONSTANT REGION GENE SEQUENCE ENVIRONMENTS IN MICE, Kenneth B. Marcu, Nancy Ann Penncavage, Julian Banerjee and Norman Arnheim, Biochemistry Dept., S.U.N.Y. @ Stony Brook, Stony Brook, N.Y. 11794.

Studies on the nucleotide sequence environments of mouse immunoglobulin heavy chain constant region genes (C_μ, C_{2b} & $C\alpha$) in the germ lines of inbred mouse strains (Balb/cJ, C571, SMJ, C57Bl/6J and C3H) have disclosed sequence variations observed by restriction enzyme digestion and Southern Blotting Analysis. The variation in the C_μ gene environment would appear to be entirely due to 5' terminal length heterogeneity. This variable sized 5' flanking sequence appears to be positioned in between a region of DNA possessing at least one J_H sequence and the C_μ gene in the Balb/c germ line. This result was obtained from genomic cloning experiments (see diagram). Four genomic clones have been isolated and each is a different size with length variation occurring precisely in the site observed for the germ lines of inbred mouse strains. Furthermore since these are the only C_μ sequences observed, these results would also suggest that only one C_μ gene is present in the mouse germ line. Analysis of F_1 heterozygotes clearly demonstrated two different sequence environments for the C_μ gene in agreement with their different inbred parental strains. Flanking sequence variations are also observed to some degree for the $C\alpha$ gene but only minimally for the C_{2b} gene.

R J_H^n(?) R 1-5 Kbp C_μ R
K B H3 B H3 H3 H3 H3 H3 B H3 K H3

379 ANTISERUM DIRECTED AGAINST AN Ig-1 LINKED MARKER IS A POLYCLONAL TRIGGER FOR T CELLS. F.L. Owen, A. Finnegan, and P.D. Gottlieb, Tufts University, Boston MA 02111 and Massachusetts Institute of Technology, Cambridge MA 02139.

An anti-T cell serum raised in allotype congenic mice recognizes the product of a new locus coding for a heavy chain-linked polypeptide found on a subpopulation of T cells. Anti-Ts^d raised in BALB/cAnN mice against selected C.AL-20 T cells reacts with a cell surface antigen in virgin animals that is found on 25% of mature thymocytes and Lyt2 bearing T cells, but not on prothymocytes, Lyt1 T cells or B cells. The antigen is restricted to strains bearing the Ig-1d and Ig-1^e heavy chain allotype haplotypes, and is expressed in the F1 animal. The antigen is unlinked in expression to the Lyt2, H-2, or kappa light chain loci. The antigen is not detected in the hematopoietic cells in the bone marrow and appears to mark only the mature peripheral pool of T cells. Minor histocompatibility differences could not account for the antigen, since C.AL-20 animals reject BALB/c skin grafts (48 days), but BALB/c animals do not reject C.AL-20 grafts (120 days). The antiserum blocks the binding of suppressor T cells to the cross-reacting idiotype for arsonate, while reagents specific for Fab, Fc and Ig were ineffective. Inhibition was achieved under capping conditions, suggesting that the new antigen is a determinant on the T cell receptor for idiotype or one which co-migrates at the cell surface with that receptor. It seems probable that the marker may represent a T cell constant region marker analagous to the Igh products on immunoglobulin. Antiserum against this marker induces *in vivo* triggering of Ts cells for a wide variety of T dependent antigens. A proliferative signal for Ts cells is suggested by the requirement for preimmunization and a lag period before antigen administration and for limiting doses of antibody to achieve suppression.

380 LYMPHOCYTE DEVELOPMENT AND PROCESSING OF MU HEAVY CHAINS, Charles Sidman, Basel Institute for Immunology, Basel, Switzerland.

Immunoglobulin-M (IgM) serves distinct and different roles in two stages of B lymphocyte development - a cell surface receptor in resting cells, and a secreted product in activated cells. The mu polypeptide chains from these two IgM populations have different apparent molecular weights in SDS-polyacrylamide gel electrophoresis, which raises the question of whether they are products of the same or separate genes. Biosynthetic studies on purified populations of lymphocytes at each of these developmental stages have shown one form of intracellular mu in common, as well as a second form unique to IgM-secreting cells. This second form unique to secreting cells appears to derive from the other (common) internal form, and to be the immediate precursor for secreted mu chains. Originally, a single, common non-glycosylated form is the ultimate mu precursor in both cell types. These results suggest that surface and secretory mu chains are not products of separate genes, but are forms of the same gene product which are processed differently for various final functions.

381 HYBRIDS OF B LYMPHOCYTE CELL LINES, W.C. Raschke, The Salk Institute for Biological Studies, San Diego, California 92138.

The objectives of our work are to determine the expression of immunoglobulin in B lymphocyte hybrids. 1) B cell lymphomas expressing surface 7S IgM, when fused with a plasmacytoma, secrete the B lymphoma IgM as a 19S pentamer. The J chain molecule responsible for pentamer assembly is undetectable in the B lymphoma parent but is present intracellularly in the plasmacytoma. Thus, complementation of the parental genomes is implied, one supplying μ-chain synthesis and the other J chain synthesis. This analysis provides a model for studying the events involved in the conversion of the B lymphocyte to immunoglobulin secretion. 2) A hamster B cell lymphoma fused with normal mouse B lymphocytes produces hybrids with surface IgM and IgD of mouse origin. The IgM and IgD molecules share the same light chain and the same idiotype. Attempts to obtain secreted IgM or IgM plus IgD from these lines by fusion with plasmacytomas failed. 3) Although most Abelson virus transformed B lymphocytes do not express immunoglobulin, some express intracellular light chain or heavy chain. The fusion of a line with intracellular κ chain with a plasmacytoma produced a hybrid which secretes the κ light chain along with the plasmacytoma heavy and light chain.

382 RETROVIRUSES AND HAEMATOPOIETIC DIFFERENTIATION, D. Frisby, I. Pragnell, D. Hughes, C. Jasmin, and W. Ostertag, The Beatson Institute, Glasgow, G61 1BD.

Combined biological studies and molecular analyses have demonstrated certain defective retroviruses which affect haematopoiesis in the mouse, each isolate containing different genetic information possibly involved in the normal differentiation of cells in this compartment. Our studies have been carried out on two murine defective transforming viruses which cause erythroleukaemia. First, the defective spleen - focus - forming virus (SFFV) of the Friend virus complex (FV-P) and second the myeloproliferative virus derived from Moloney sarcoma virus MPV(MSV). Both cloned complexes contain the same LLV-F helper virus and a defective transforming virus, which in the case of MPV(MSV) is also a sarcoma virus. Specific complementary DNAs to both defective components, free of helper virus related sequences, have been purified. $cDNA_{SFFV}$ does not hybridise to MPV(MSV) RNA but does contain xenotropic SFFV related sequences, conversely, $cDNA_{MPV}$ contains neither FV-P nor xenotropic virus related sequences. It carries all MSV specific sarc sequences and, in addition, new specific sequences which may be of cellular origin. Restriction enzyme analysis of virus-specific DNA from Friend cells and from $SFFV^{+}LLV^{-}$ non-producer cell-lines together with analogous studies on MPV(MSV) non-producer cells has allowed preliminary mapping of the DNA proviruses of both defective viruses and the LLV-F helper in cells representing different stages of differentiation. Current studies involve the molecular cloning of DNA from DBA/2 mice, Friend cells of DBA/2 origin and SFFV nonproducer cells, also of transformed NRK non-producer fibroblasts.

Control of the Cell Cycle

383 CELL CYCLES NEED TWO RANDOM TRANSITIONS, Robert F. Brooks, Dorothy C. Bennett and James A. Smith, Imperial Cancer Research Fund, P.O. Box 123, Lincoln's Inn Fields, London WC2A 3PX, U.K.

Differences between the generation times of sibling cells are distributed exponentially indicating the existence of a step in the cell cycle which takes place at random. The distribution of generation times, however, is not perfectly exponential demonstrating an additional source of variation that nevertheless affects pairs of sibling cells identically. As a result, the cycle times of siblings are correlated. This correlation and the overall distribution of generation times can be predicted quantitatively by the existence of a second random transition in the cell cycle which, except at very low growth rates, takes place in the parental cell cycle. The two random transitions are separated by a lengthy process (equivalent to the minimum cell cycle time) which, since it spans cell division, is out of phase with the conventional cycle (M-G_1-S-G_2-M). Instead, it may be tied to the centriole cycle.

384 CHARACTERIZATION OF CELL LINES ESTABLISHED WITH MUTATIONS IN THE EGF PROLIFERATIVE RESPONSE, Joseph M. Sorrentino and Harvey R. Herschman, Department of Biological Chemistry and Laboratory of Nuclear Medicine and Radiation Biology, U.C.L.A. School of Medicine, Los Angeles, California 90024

Cellular mitogenesis consists of a complex and, as yet, primarily unknown series of events. Its study is facilitated considerably by the use of defined "growth factors" whose mechanism of action can be studied. We have been utilizing epidermal growth factor (EGF) to characterize the proliferative response in Swiss 3T3 mouse embryo cells. EGF addition will stimulate quiescent cultures of 3T3 cells to reinitiate proliferation. In view of this, one approach to the study of mitogenesis is to establish variant cell lines which no longer manifest a proliferative response to EGF and then to characterize these cells as to whether they (i) continue to respond to other known mitogenic compounds, (ii) have any alterations in either the quantity or affinity of EGF receptors, and/or (iii) are blocked in biochemical or physiological steps distal to receptor occupancy in the proliferative response. We have established such cell lines, and the delineation of the characteristics of one variant, 13_{6A}, which possesses EGF binding and degradation ability - but does not have a mitogenic response to epidermal growth factor - is the basis of this report.
(Supported by NIH grant GM- 24797 and DOE Contract DE AM03 76 SF00012 (HRH) and by NCI Grant 5T32CA9030 (JMS).)

385 RELATIONSHIP OF ANCHORAGE AND SERUM DEPENDENCE WITH CENTRIOLE CYCLE IN BALB/C-3T3 AND SV40 TRANSFORMED BALB/C-3T3 CELLS, Robert W. Tucker[*], Arthur B. Pardee[+], Judah Folkman[++], [*]Johns Hopkins Oncology Center, Baltimore, MD, [+]Sidney Farber Cancer Institute, and [++]Children's Hospital Medical Center, Boston, MA.
Balb/c-3T3 cells become quiescent in low serum or at high cell density with unduplicated, ciliated centrioles (Cell 17: 527, 1979). Serum, but not SV40, produces an early centriole deciliation associated with early mitogenic events. Crowded cell cultures when wounded also exhibit the same changes in the centriole and DNA synthesis cycles as that produced by serum. Crowding of cells affects DNA synthesis primarily through an affect on cell spreading or anchorage (Nature 273: 345, 1978), so that the similarities of serum-starved and crowded cultures may indicate that serum and anchorage affect similar events in the cell cycle. When prevented from spreading on poly (HEMA) substrata of low adhesivity, Balb/c-3T3 cells and other non-neoplastic cells grew slowly and eventually stopped, whereas the growth of SV40 transformed Balb/c-3T3 and various neoplastic cells slowed comparatively less, and did not stop. Rounded non-neoplastic cells also became increasingly refractory to serum stimulation. In contrast, SV40 transformed Balb/c-3T3 and some neoplastic cells on poorly adhesive substrata changed their sensitivity to serum only slightly. Thus events produced by SV40 bypass early centriole events produced by serum stimulation, and uncouple the effect of cell shape or cell spreading on growth stimulation by serum. Centriole events may be important in the coupling of anchorage and serum effects on cell growth.

386 MAPPING THE MITOTIC CLOCK BY PHASE PERTURBATION, R.R. Klevecz, G.A. King and R.M. Shymko, City of Hope National Medical Center, Duarte, CA 91010

A four hour periodicity has been observed within the cell cycle of animal cells. Synchronized V79 cells perturbed by serum, heat shocks, ionizing radiation or cAMP phosphodiesterase inhibitors at half-hour intervals through a modal 8.5 hour cell cycle display a characteristic biphasic pattern of advances and delays in subsequent cell divisions. Although the value and amplitude of phase changes varied from agent to agent, the saw-toothed pattern of phase resetting was similar in all instances. We are led to consider a model incorporating a two-component oscillator with a threshold for the initiation of DNA replication and mitosis. The model predicts desynchronization of cell populations by perturbations of appropriate phase and intensity. Desynchronization experiments to date are most consistant with a relaxation-type limit cycle oscillator. If oscillator variables do not follow a fixed trajectory but are normally distributed astride that trajectory, then the resulting distribution of generation times is exponential, but quantized within the exponential envelope.

387 RESTRICTION POINT MAPPING OF THE UTERINE G_1 PERIOD WITH A SHORT-ACTING ESTROGEN,
Gary Stack and Jack Gorski, University of Wisconsin, Madison WI 53706.
Estradiol-17β (17β-E_2) administration *in vivo* stimulates by 5-10 fold ^{3}H-thymidine triphosphate incorporation into DNA of isolated uterine nuclei after a 15 hr lag period. In contrast, the short-acting estrogen, estradiol-16α (16α-E_2), has no effect on DNA synthesis while stimulating maximally other responses assayed at 4 hr. Although there is no impairment in the ability of 16α-E_2 to translocate cytoplasmic estrogen receptors to the nucleus, receptors bound to 16α-E_2 are cleared from the nucleus in 3-4 hr compared to about 12 hr with 17β-E_2. This suggests a requirement for long-term nuclear receptor retention for DNA synthesis induction. If 16α-E_2 is administered via five lug injections at 3 hr intervals in order to prolong tissue estrogen retention time, DNA synthesis is stimulated to the same extent as with 17β-E_2, but with a longer G_1 period. Priming the uterus with one injection of 16α-E_1 shortens by 3-4 hr the lag between subsequently administered 17β-E_2 and DNA synthesis. The memory of this priming is stable for 20 hr but decays thereafter, suggesting that molecules which turn over slowly are involved. When 3 priming injections of 16α-E_2 are given at 3 hr intervals, the 2 additional injections each further shorten the lag but to a lesser extent than the first. A ratchet model for the uterine G_1 period is proposed in which cells must pass through at least three restriction points requiring estrogen. Each injection of 16α-E_2 pushes uterine cells a small distance into G_1, while a single injection of 17β-E_2 allows complege G_1 transversal. (This work was supported in part by NIH Grants HD 08192 and GM 07215.)

388 CONTROL OF MOUSE MYOBLAST DIVISION AND DIFFERENTIATION BY MITOGENS: COMMITMENT DURING G_1 OF THE CELL CYCLE AFTER MITOGEN WITHDRAWAL.
Tom Linkhart, Chris Clegg, and Steve Hauschka, Dept. of Biochemistry, Univ. of Washington, Seattle WA 98195.
Removal of mitogens from a permanent line of mouse myoblasts leads to irreversible withdrawal from the cell cycle and commitment to terminal differentiation even if mitogens are fed back (Linkhart et al. J.C.B. 79:25a; 83:24a). Kinetics of withdrawal from the cell cycle were determined by thymidine pulse labeling. After a 2-3 hr lag, during which cells traverse the cycle normally (G_1=2 hrs, S=7.5 hrs, G_2+M=3 hrs) committed cells cease entering S and the pulse labeling index drops to zero by 10 hrs. Commitment kinetics were determined by withdrawing mitogens for increasing times followed by refeeding mitogens plus ^{3}H-Thymidine for another 18 hrs; committed cells were defined as those which did not label after refeeding. After the 2-3 hr lag the % committed cells increased until all were committed within one cell cycle time. Committed cells elaborate acetylcholine receptors (AChR) and fuse to form myotubes even in the presence of mitogens. The kinetics of withdrawal support a simple model for mouse myoblast commitment: after a 2-3 hr lag cells in G_1 commit and do not enter S; cells in M, G_2, and S commit when they cycle into G_1. The model is supported by the failure of mitotically synchronized cells to enter S when plated into mitogen-deficient medium (all were AChR positive by 24 hrs.) while mitotic cells plated into complete medium all entered S (none became AChR positive). A purified mitogen, Fibroblast Growth Factor stimulates mouse myoblast proliferation and prevents commitment. These studies suggest that the presence or absence of a mitogenic signal during G_1 determines whether mouse myoblasts continue to proliferate or commit to terminal differentiation.

389 GLUCOCORTICOID STIMULATION OF CELL PROLIFERATION: A CELL CYCLE DEPENDENT RESPONSE,
Vincent J. Cristofalo, Gary Grove, Cathy Finlay, Jane Miller, and Bryon Rosner, The Wistar Institute, Philadelphia, PA 19104
The proliferation of WI-38 cells is stimulated by physiological concentrations of glucocorticoids. This stimulation is dependent on cell-steroid interaction which is cell cycle dependent and which is restricted to the G_0-G_1 transition period when peak specific binding of glucocorticoids is observed. This cell cycle dependence of steroid-cell interaction can be demonstrated both in confluent quiescent monolayers stimulated to proliferate by the addition of fresh medium and in cultures newly subcultivated at low density (1 x 10^4 cells/cm^2). The addition of glucocorticoids to exponentially growing cultures 24 or more hours after seeding did not cause stimulation. The direct result of the interaction of the cell with the steroid is the production of a factor into the medium which in turn appears to block density dependent inhibition of cell proliferation. Multiple refeedings with "glucocorticoid conditioned" medium containing the putative growth factor resulted in a major extension of the logarithmic growth period and multilayering (3-4 monolayer equivalents) with saturation densities 2-3 times that of multiple refed controls. Our data suggest that the hydrocortisone-induced "growth factor" may act by maintaining cells in the proliferation "competent state" and thus continually responsive to serum mitogens.
Supported by NIH Grant AG-00378.

390 NERVE CELL COMMITMENT OCCURS DURING A RESTRICTED PORTION OF THE CELL CYCLE IN HYDRA ATTENUATA, Hans R. Bode, Bruce A. Baca and Marcia S. Yaross, Department of Developmental and Cell Biology, University of California, Irvine, CA 92717
Nerve cells in hydra differentiate from the interstitial cell, a multipotent stem cell. Decapitation elicits a 10- to 20-fold increase in the fraction of the interstitial cells which undergo nerve cell differentiation in the tissue which forms the new head. To investigate when during the cell cycle nerve cell commitment can be stimulated, hydra were pulse-labelled with ^{3}H-thymidine at times from 18hr before to 24hr following head removal; the resulting cohorts of labelled interstitial cells were in the various phases of the cell cycle at the time of decapitation. Commitment to nerve cell differentiation within a single cell cycle ($\sim$24hr) was observed only in those cohorts which were at least 3 hours before the end of S-phase (12hr) at the time of head removal. The maximum time required for head removal to result in the stimulation of nerve cell differentiation was also measured. At times 3 to 14hr after head removal, hydra were pulsed with ^{3}H-thymidine. The regenerating cells were then transplanted to aggregates of unlabelled hydra cells and the differentiation of labelled nerve cells assayed. The interval required between head removal and transplantation to obtain increased labelled nerve cell differentiation was found to be between 3 and 6 hours. These results indicate that the last point at which commitment to nerve cell differentiation occurs is no later than three hours past the S/G_2 phase boundary. (Supported by NIH grants HD08086 to HRB and HD05705 to MSY.)

391 INDUCTION OF CELLULAR DNA SYNTHESIS BY THE SV40 A GENE, Joanna Floros, Gerald J. Jonak, Norbel Galanti and Renato Baserga, Temple University School of Medicine, Philadelphia, Pa. 19140.
ts13 and tsAF8 are temperature-sensitive (ts) mutants of BHK cells that arrest in G_1 at the nonpermissive temperature (npt) of 39.5°C - 40.6°C. When made quiescent by serum deprivation, subsequent serum stimulation is incapable of overcoming the ts block in G_1. Because both cell lines are refractory to SV40 infection we cloned the SV40 A gene in the pBR322 plasmid and we microinjected it by the Graessmann technique in both ts13 and tsAF8. Cells microinjected with the cloned SV40 A gene entered DNA synthesis even at the npt. mRNA from the SV40 A gene, isolated by hybridization to viral DNA, also induced DNA synthesis when microinjected into ts13 and tsAF8 cells, at both pt and npt. We are now identifying the DNA sequence in the SV40 A gene that is both necessary and sufficient for the induction of cellular DNA synthesis.

392 POSITIVE AND NEGATIVE CONTROL OF MAMMALIAN CELL PROLIFERATION: EVIDENCE FROM CELL FUSION STUDIES, Gretchen Stein and Rosalind Yanishevsky, Univ. of Colorado, Boulder, CO
When young human diploid cells (HDC) are deprived of serum, they enter a nonreplicative quiescent state. We investigated the nature of this quiescent state by fusing quiescent HDC to either cycling HDC, chemically transformed HDC, T98G glioblastoma cells, HeLa cells or SV40 transformed HDC (SV40-HDC). HeLa and SV40-HDC induced DNA synthesis in the quiescent nuclei in heterodikaryons, i.e., HeLa and SV40-HDC were dominant. The other three types of replicative cells were recessive: they did not induce DNA synthesis in the quiescent nuclei, and their own nuclei were inhibited from entering S phase. However, ongoing DNA synthesis was not inhibited in the replicative nuclei. These data suggest that serum-deprived quiescent HDC may contain a diffusible inhibitor that prevents entry into S phase. The same pattern of dominance and recessiveness, was observed when senescent HDC were fused to the identical series of replicative cells. The parallel behavior of these two types of nonreplicative cells suggests that they may inhibit entry into S phase by the same mechanism.

Why are HeLa and SV40-HDC dominant over senescent and quiescent HDC when other transformed cell lines, including "fully transformed", tumorigenic cell types, are recessive? We suggest that HeLa and SV40-HDC have gained an inducing factor, e.g., through viral transformation. Furthermore, we suggest that the recessive transformed cell lines have lost all or part of their normal mechanism for control of cell proliferation, e.g., through mutation. Our studies with transformed cells of known etiology support this hypothesis: chemically transformed HDC and radiation transformed HDC are recessive, whereas SV40-HDC and adenovirus transformed HDC are dominant. This work was supported by NIH grant AG00947.

393 GENETIC ANALYSIS OF THE PRESENCE AND ABSENCE OF G1. R. Michael Liskay, MCD Biology University of Colorado, Boulder, Colorado 80309.

Whereas most mammalian cells in vivo and in vitro cycle with a G1 interval ($G1^+$ cells), certain extraordinary cells in vivo, e.g. blastomeres, and several in vitro lines, e.g. V79-8 "fibroblasts," cycle without a measurable G1 ($G1^-$ cells). The $G1^+$ and $G1^-$ phenotypes have been investigated in our laboratory utilizing somatic cell genetic techniques. The $G1^-$ phenotype always behaves dominantly in intraspecific cell hybrids. Recessive $G1^+$ mutants of the V79-8 $G1^-$ line have previously been isolated and define at least four $G1^+$ complementation groups. Complementation is indicated by the production of $G1^-$ hybrids from two $G1^+$ cells. These findings suggest that the $G1^-$ state is the "prototype" for cycling cells and that each $G1^+$ mutant represents a different deficiency in a function necessary for being $G1^-$. Surprisingly, four different $G1_+$ established cell lines of C. hamster, e.g. CHO and DeDe, have been found to define three $G1^+$ complementation groups. This suggests that different naturally-occurring $G1^+$ cells can express a measurable G1 for different reasons, e.g. different deficiencies. Recently, complementation tests have been performed between these $G1^+$ established lines and early-passage fibroblast-like cells from the lung and liver of a single newborn hamster. The results of these complementation tests show that the basis for the G1 interval of the "lung" cultures is not identical to that of the "liver" cells. Results further suggest that the G1 basis of a particular cell does not change detectably even after extended periods of time in culture ("transformation"). It is concievable that a cell's G1 basis is related to that cell's developmental state.

394 DIFFERENTIAL FLUORESCENCE DETECTION OF CYCLING AND NONCYCLING CELLS (G_o?), Paul K. Horan,* Katharine A. Muirhead,* James E. Gill,* Alan Waggoner+, *University of Rochester Medical Center, Rochester, NY 14642 and +Amherst College, Amherst, MA 01002

Analysis of cell cycle distributions has become a major technique in cell biology research. Classical staining techniques for "DNA content" make possible the determination of the percentage of the cells analyzed which are in the G_1/G_o-phase, S-phase, or G_2 + M-phase of the cell cycle. These methods alone are incapable of distinguishing G_o cells from G_1 cells.

We report a new staining method which stains cycling cells at least five times more intensely than noncycling cells. These procedures (including analysis) can be carried out on cells in less than 20 minutes and do not change the cells ability to exclude Trypan Blue. With these methods we have demonstrated that plateau phase cells (noncycling cells) can be stimulated into the "cycling state" in less than three hours after diluting cells into fresh growth medium. Furthermore, cycling cells can be shifted to the plateau state by concentrating the culture; resulting in a shift in fluorescence intensity.

Similar findings were obtained using a human fibroblast cell culture. Confluent cultures were wounded by scraping a strip of cells off the coverslip. At the wound margins cells begin cycling to grow into the vacant regions. After an appropriate incubation at 37° C., the coverslips were stained and the fluorescence intensity measured on cells in the wound margin and cells growing in the confluent areas. As stated above, "cycling cells" in the wound margins fluoresce with an intensity 4-6 times greater than "noncycling cells" in the confluent areas.

395 CELL CYCLE RE-ENTRY OF QUIESCENT MAMMALIAN NUCLEI FOLLOWING HETEROKARYON FORMATION, Robert A. Schlegel and W. Edward Mercer, The Pennsylvania State University, University Park, PA 16802

When 3T3 mouse fibroblasts are made quiescent by serum deprivation and are then fused with AF8 hamster fibroblasts synchronized in G1/S by hydroxyurea, the nuclei of binucleated heterokaryons which are formed enter S-phase asynchronously in media containing low levels of serum. The AF8 nuclei of these biphasic heterokaryons enter S-phase shortly after fusion, as do the AF8 nuclei of homokaryons in the same culture. In contrast, the nuclei of the biphasic heterokaryons which have been contributed by quiescent 3T3 enter S-phase only after a lag following fusion. This suggests that the quiescent nucleus within the heterokaryon is stimulated by factor(s) from the more advanced G1/S-phase cell to re-enter the cell cycle in the absence of serum. In contrast to factors which induce the immediate synthesis of DNA, these factors may be those responsible for the transition of a cell from a non-proliferating to a proliferating state.

396 COMPUTER SIMULATION OF THE CELL KINETICS OF GROWTH CONTROL, LaRoy N. Castor, The Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA 19111

Two computer programs are described for simulation of the growth, cloning, and labeling characteristics of cultured cells during continuous growth, during growth accompanied by sensescence or transition to a differentiated state, and after a change in concentration of serum or other growth regulating factors. Up to 1000 cell lineages are followed throughout a culture interval, with subcultivation from randomly selected cells. Initial results have used a cell cycle model with normally distributed reciprocals of the lengths of the G1 phase (G1 rates), normally distributed times of passage through the remainder of the cycle, and a probability that the mean G1 rate will be inherited at some reduced value (J. Cell Biol., 83:13a, 1979). This model leads to doubling times, proportions of non-cycling cells, and distributions of clone sizes resembling those of a senescing cell population. Statistical correlation coefficients between the generation times of siblings and between those of mother and daughter cells vary widely with the growth history of a culture, suggesting that these coefficients are unreliable indicators of any physiologically meaningful growth parameter. The programs are written in Fortran IV-Plus for a relatively small (32,000 word) computer. They can be supplied to other investigators on magnetic tape and are readily modified for other cell cycle models and other input and output data. Auxiliary programs using a Statos Printer/Plotter are available for high-resolution plotting of the output data. (Supported by NIH grants CA-06927, CA-22780 and CA-24961 and by the Commonwealth of Pennsylvania.)

397 EVIDENCE FOR A UNIQUE G_1 ARREST POINT FOR CELL DIFFERENTIATION, Dagne L. Florine and Robert E. Scott, Section of Experimental Pathology, Mayo Clinic, Rochester, Minnesota.

Differentiation of normal cells is associated with growth arrest in the G_1 phase of the cell cycle. To determine if such growth arrest occurs at a unique point in the G_1 we compared 3T3 T proadipocytes which were spontaneously arrested prior to differentiation and 3T3 T cells G_1 arrested by serum or nutrient deprivation. Maximum differentiation of confluent cells occurred following culture for 16 days in medium containing 30% FCS and insulin (50 μg/ml). Three to five days prior to the expression of the differentiated phenotype >90% of the cells spontaneously growth arrested in G_1. Such cells could be induced to differentiate into adipocytes by culture in media containing nonmitogenic differentiation promotors, i.e., 30% platelet poor serum plus insulin or $PGF_{2\alpha}$. By contrast, growth arrest in G_1 at the serum arrest point (G_0) under a variety of conditions did not result in differentiation nor did subsequent culture in nonmitogenic promotors of differentiation. Similarly, cells arrested at the nutrient arrest point (G_N) by culture in isoleucine deficient medium did not differentiate nor did cells subsequently cultured in nonmitogenic differentiation promotors either with or without added isoleucine. These data suggest that growth arrest in G_1 prior to differentiation does not occur at G_0 or G_N but rather at a unique G_1 arrest point which we have designated G_D. G_D arrested cells differ from G_0 and G_N cells not only in their ability to differentiate but also because G_D cells are not stimulated to proliferate by serum, nutrients, or various growth factors and are more highly sensitive to cAMP-induced cytotoxicity. We propose that G_1 growth arrest at G_D serves to couple control of cell growth and differentiation in normal cells and that malignant transformation may be associated with a defect in control of G_D arrest.

398 DOES THE VARIABILITY OF THE CELL CYCLE RESULT FROM ONE OR MANY CHANCE EVENTS?, Arthur L. Koch, Department of Biology, Indiana University, Bloomington, IN 47405

It is argued that in almost all populations, a small subfraction of the cells occur which grow more slowly than do the majority. It is shown that 1) γ_1, the skewness of the distribution of cell ages at division, 2) ρ_{md}, the correlation coefficient of the ages at division of mother and daughter cells, and 3) the shape of the β-curves are all highly influenced by a small slow growing subfraction. This means that the process which times the cell division cycle of the bulk of the cells cannot be inferred from measurements of these quantities unless the presences of a small subpopulation of more slowly growing cells can be ruled out. Thus, one can not test between single event and multiple event timing.

If a few percent of more slowly growing cells are removed from the experimental population, the transition probability model of Smith and Martin fails to fit the experimental data. On the other hand, the growth controlled model of Koch and Scheachter can be made to fit the statistical parameters of ages at division of a variety of organisms when a 2 - 4% population of slower growing cells is subtracted out. The postulates on which the latter model is based have been established independently of statistical considerations for one species, Escherichia coli. It proposed that the measurement of the distribution of cell properties at division, the equality of the division process, the growth kinetics of single cells throughout the cycle will lead to a more critical understanding of the mechanisms controlling the division cycles of other cells than the study of γ_1, ρ_{md}, or the shape of β-plots.

399 AN ALTERNATIVE VIEW OF THE G0 and G1-ARREST PHASES OF THE EUCARYOTIC CELL CYCLE,
Stephen Cooper, Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, Michigan, 48109.

I have previously presented a unified model of the cell division cycle for both prokaryotes and eukaryotes. In this model no specific "G1 functions" occur during the G1 period, which is merely part of a larger period for the preparation for DNA synthesis which began at the previous initiation of DNA synthesis. A G1 period was postulated to exist merely because the doubling time of the cells is greater than the sum of the S and G2 periods (Cooper, S. (1979), Nature, 280:17-19). I now extend this model to explain the postulated G0 or G1-arrest states of eukaryotic cells. These cells have supported the idea that there are specific points in the G1 period at which cells can be growth arrested. In contrast to the proposal that there are such resting or stationary phases in which cells can accumulate, I will reanalyze the available data to show that the cells which are found resting in G1 (defined as having a G1 content of DNA) are not in the same state as cells in the normal G1 period of cycling cells. This is because there is a dissociation of the synthesis of initiation potential and the actual synthesis of DNA in these resting cells. The results on resting cells can be explained without having any event uniquely occurring in the G1 period. Also, there is no "G0" period or state or "long G1" in which cells are found to be resting. The application of this model to the understanding of the immune proliferative response, mitogenesis, and neoplastic growth will be described.

400 ALTERATIONS IN DNA SYNTHESIS IN MOUSE LIVER CELLS CAUSED BY THE TUMOR PROMOTOR 12-O-TETRADECANOLYPHORBOL-13-ACETATE(TPA).
James C. Bartholomew, Ann Hughes and Krishnakali Das, University of California, Berkeley, CA 94720.

We have been studying the effects of TPA on the movement of cells around the cell cycle. We monitored cell cycle position by flow cytometry as well as 3H-thymidine incorporation into DNA. When TPA was added to the culture medium(1.0 ug/ml) of growing liver cells, the first observed change in cell cycle distribution was a block in the movement of cells out of G_1 into the beginning of S. This effect was seen by 2 hours after adding the compound to the medium. This early block in movement out of G_1 eventually results in a depletion of cells in S. The depleted S was refilled with cells moving from G_1 beginning at 10 hours after TPA addition. This renewed movement of cells through S was partially synchronous. When quiescent cells are stimulated by serum in the presence of TPA, the kinetics of cell cycle movement depends on when relative to serum stimulation the TPA is added. When TPA is added at the time of serum stimulation the wave of cells moving from G_1 to S is more synchronous than controls. There is no evidence that more cells are caused to move through the cycle in the presence of TPA than in its absence. Addition to TPA just as the cells begin entering S again causes the cells in G_1 to delay their entrance into S for approximately 10 hours. These studies indicate that TPA causes an increase in synchrony of cycling cells by increasing the probability that cells will enter G_0 from G_1, and that once cells are in G_0 they reenter the cell cycle with the characteristic long lag.

401 REGULATION OF CELL CYCLE INITIATION IN YEAST BY α-FACTOR,
V.N.Smirnov,L.V.Lizlova, A.A.Minin, D.D.Bespalova, M.I.Titov and G.P.Samokhin, USSR Research Center of Cardiology, Petroverigsky Lane IO, Moscow IOI837, USSR.

In the study of molecular mechanisms controlling eukaryotic cell cycle the compounds which specifically affect various stages of cell cycle can be of value.In this respect α-factor, sexual pheromone of yeast Sacch.cerev.,can be considered as natural regulator of cell cycle initiation in simplest eukaryote. α-factor specifically and reversibly arrests a-cells of yeast at the point "start" of G_I before cell cycle initiation. Using biologically active synthetic analog of α-factor (N-Trp,Arg^7)-α-factor (TA-αfactor) it was found that this compound exerts its effect at "start" by lowering the rate constant of cell cycle initiation.In terms of "transition probability" concept it means that α-factor lowers probability of cell cycle initiation per unit of time for each cell and does not act by "all or none" principle.The initiation rate changes abruptly with lag period of 30 and 40 min after shift up or -down in α-factor concentration, respectively.The difference in two lag periods suggests the existence of two independent mechanisms of α-factor effect on transition probability which may differ by relaxation intervals and may cross before "probability generator." It is likely that no cooperative interactions are involved in either mechanism, for the dependence of relative constant of cell cycle initiation on TA-α-factor concentration is characterized by Hill coefficient n=I.OI $\pm$0.05 (m$\pm$sem).More than IO-fold inhibition of protein synthesis by actidione has no effect on the position and the shape of the dependence curve. The latter implies that at least one of the mechanisms does not involve the induction of a specific protein (initiation inhibitor).

402 MITOGEN-ACTIVITY B-LYMPHOCYTES REQUIRE THE CONTINUOUS PRESENCE OF THE GROWTH PROMOTING MITOGEN TO PASS THROUGH SUCCESSIVE CELL CYCLES,

Waldemar Lernhardt and Fritz Melchers, Institute for Immunology, 4005 Basel, Switzerland.

Small resting B lymphocytes are activated asynchronously by the mitogen lipopolysaccharide. A 40h stimulated B-cell population therefore, contains cells in all stages of the cell cycle. Synchronization was achieved by separating the cells according to size by velocity sedimentation at unit gravity. Since the size of B lymphocytes increases as they proceed through the cycle, a given size of an activated lymphocyte represents a given phase in its cell cycle.

Cells in various phases such as early G_1, late G_1, S, and G_2 phases were recultured with and without mitogen. These cells at various stages of the cell cycle continued to divide synchronously for several divisions in the presence of mitogen. B-cell blasts recultured without mitogen completed the initiated cycle and divided, if they had proceeded at least two hours into G_1-phase after mitosis. B cell blasts in the first two hours after mitosis did not proceed into S-phase. Such "G_1cells" kept secreting IgM for several days.

We conclude that the continued presence of mitogen is not needed for IgM secretion from activated B-cell blasts. Mitogen is required early in G_1 to stimulate the cell into the next round of division.

403 THE EFFECTS OF TUMOR PROMOTORS ON CELL STRUCTURE, CELL DIVISION AND DIFFERENTIATION IN MACROPHAGES, S.R. Dienstman*, W. Corry*, J. Hamilton°, V. Defendi*, New York University Medical Center, New York* 10016, Sloan Kettering Institute, New York°

Two physiologically different populations of primary, GO, macrophages have been treated with tetradecanoyl phorbol acetate (TPA), resident mouse peritoneal cells (NpMAC) which do not divide when treated with the native growth hormone, MGF (= CSF) and starch activated cells (STpMAC) which do replicate DNA and divide with MGF treatment. TPA causes STpMAC but not NpMAC to divide. Cycle kinetics are similar to normal MGF induction. However both kinds of pMAC change shape in the same way, spreading flat upon TPA addition. Cell-substrate adhesiveness, tested in a centrifugation assay, is increased in TPA-treated cells. Shape and adhesion changes are antagonized by cytochalasin. TPA, like MGF, induces plasminogen activator synthesis and secretion. Unlike its presumed role in transformation, TPA is a differentiation promotor on pMAC cells, and the cytological and synthetic changes are independent of mitosis.

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404 ENIGMATIC ERYTHROPOIESIS or WHERE DO ALL THE CELLS GO? Michael C. Mackey, Department of Physiology, McGill University, Montreal, Quebec, Canada H3G 1Y6

An extensive survey of measured steady state DNA synthesis (S) phase [labelling index (f_L) and synthesis time (t_S)] and mitotic (M) phase [mitotic index (f_M) and mitotic time (t_M)] data for the recognizable (pro-, basophillic-, and polychromatic erythroblasts) proliferating erythroid precursors (PEP) in humans, guinea pigs, and rats has revealed a striking observation: $(f_L/t_S) > (f_M/t_M)$ within each morphological category (MC). Thus, within each MC, the flux of cells through S is greater than through M. The same discrepancy has been pointed out by Rubinow [J Math Biol (1975), 1 187] for proliferating myeloid precursors. If these data are to be believed, then we must explain what happens to the cells that went through S in a given MC but which did not make it through M in that MC. One possibility is that they die, but the data are incompatible with this hypothesis. A second possibility is that PEP may differentiate between MC in G1 as well as in G2. The steady state human erythroid data are completely consistent with this hypothesis, and allows an exact calculation of all relevant PEP cellular populations and the fluxes between them. Using these values calculated from the steady state data I have computed the expected time dependent labelling index, grain count, and percent labelled mitoses for the human PEP, and find the computed values consistent with previously published human data. These, and other considerations, will be presented in detail.

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405 FLOW-CYTOMETRIC ANALYSIS OF CHICK ERYTHROPOIESIS. Richard M. Allen and David Bloch, Cell Research Institute & Botany Department, University of Texas, Austin,TX 78712
Double fluorochroming of hemoglobin (by removal of Fe; Granick and Levere,J.Cell Biol. 26, 167,1965) and DNA (by acriflavin-Feulgen staining) permits simultaneous measurement of both substances by flow-cytometry. In erythropoiesis a precursor stem cell gives rise to a commited erythroblast which divides 6 times while synthesizing hemoglobin (Weintraub et al, J. Cell Biol. 50, 562,1971). Hemoglobin, used as an index of differentiation permits the resolution of the cells belonging to the different generations, whose cell cycle kinetics can be studied by analysis of DNA distributions. Erythropoiesis is used in this study as a model to aid in our understanding of the process of clonal attenuation.

406 VARIANTS OF 3T3 CELLS LACKING MITOGENIC RESPONSE TO THE TUMOR PROMOTER TETRADECANOYL PHORBOL ACETATE (TPA), E. Butler-Gralla and H. Herschman, UCLA, Los Angeles CA 90024
One of our laboratory's basic aims has been to understand the series of events by which a previously non-dividing cell enters the cell division cycle. Our approach has been to isolate cell lines that do not respond to specific mitogens, and to determine what lesion causes the nonresponsiveness. TPA is a potent mitogen for 3T3 cells, as well as being a tumor promoter. Four independent variants of 3T3 cells that do not divide or reach an increased saturation density in response to TPA have been isolated. All variants retain response to elevated levels of fetal calf serum. Three variants retain response to Epidermal Growth Factor (EGF); one does not. This is interesting in view of the reported effects of TPA on EGF binding. EGF binding studies are being done on all the cell lines. The cell line that does not respond to EGF has no measurable EGF binding. The others bind EGF in varying amounts. In these lines, EGF binding in the presence and absence of TPA has been measured at 37°. In all cases, TPA decreases the affinity of the EGF receptor for EGF to approximately the same degree as it does in the parent 3T3. Thus we have isolated cell lines that retain some response to TPA (i.e. modulation of EGF binding), but have lost the mitogenic response. These variants will be useful for the study of the nature of the mitogenic response, the mechanism of tumor promotion, and the interrelation between these two phenomena.

H-2 Surface Antigens and Lymphocyte Communication

407 SPECIFICITY AND ACTIVITY OF H-2 UNRESTRICTED ANTI-Qa/Tla CYTOTOXIC EFFECTOR CELLS, James Forman and Jo Ann Keene, Department of Microbiology, UTHSCD, Dallas, TX. 75235.
The specificity and activity of anti-Qa/Tla specific H-2 unrestricted cytotoxic T cells were analysed in secondary CML responses. A strain effector cells, sensitized in vivo and boosted in vitro with (congenic) A.Tla^b lymphoid cells, lysed target cells from strains with differing H-2 haplotypes but all sharing Qa-1^b/Tla^b alleles; whereas, target cells from strains with Qa-1^a/Tla^a were not. When B6.Tla^a animals were primed in vivo and challenged in vitro with (congenic) B6 lymphoid cells, no cytotoxic effector cell activity was generated. However, if B6.Tla^a animals were primed in vivo with (noncongenic) A.BY lymphoid cells and then rechallenged in vitro with either A.BY or B6 stimulator cells, cytotoxic effector cells were generated that preferentially lysed target cells from strains with Qa-1^b/Tla^b irrespective of their H-2 haplotype. This suggests that factors in addition to Qa/Tla may play a role in the generation of anti-Qa/Tla effector cell activity. When B6.Tla female animals were primed in vivo with stimulator cells from B6 males and rechallenged in vitro with either B6 male or female lymphoid cells, anti-Qa-1^b/Tla^b effector cell activity was generated. On the other hand, if B6.Tla^a female animals were primed with B6.Tla^a male cells mixed with B6 female cells no anti-Qa/Tla effector cell activity occurred. Therefore, while a limited number of antigenic factors (H-Y?) in addition to Qa/Tla allows for anti-Qa/Tla priming in vivo, both Qa/Tla antigens and these factors have to be expressed on the same cell.

408 A GENE ON THE X-CHROMOSOME REGULATES THE MEMBRANE EXPRESSION OF IA.W39, A NEW B CELL DIFFERENTIATION ANTIGEN, Brigitte T. Huber and David A. Thorley-Lawson, Tufts University SChool of Medicine, CRC, Boston, MA 02111 and SFCC, Harvard University School of Medicine, Boston, MA 02115.

We have recently defined a new private specificity for I-A, Ia.W39, which is selectively expressed on 50% of splenic B lymphocytes in adult mice of the I-A^b type, whereas bone marrow cells, thymocytes and peripheral T cells of these mice are negative. Ia.W39 is absent from B cells in adult mutant mice of the I-A^b type, carrying the defective xid gene, and in newborn normal mice, whereas the B cells of both express conventional Ia antigens, coded for by a gene(s) in the I-A region. - Immunoprecipitation of [^{35}S]-methionine labelled spleen cell lysates with anti-W39 serum gave one specific band on SDS-PAGE, which run together with the β chain of the conventional Ia molecule. No α chain was observed, but it may not label strongly with the [^{35}S]-methionine. In ontogeny studies, we found that this specific band was first detectable in a 16 day old animal, and that its intensity increased until the mouse reached adult age (> 3 weeks), whereas no quantitative nor qualitative difference in the precipitation pattern of the conventional Ia molecule in newborn and adult animals was seen. - Surprisingly, immunoprecipitation with anti-W39 of [^{35}S]-methionine labelled spleen cell lysates from the mutant mice gave the same specific band, although the intact B cells from these mice could not absorb out the W39 specificity from the antiserum. - We conclude, therefore, that W39 is synthesized in the cytoplasm of a subpopulation of B cells, and that the xid gene controls its membrane expression later on in ontogeny.

409 ARE ALLOGENEIC AND ASSOCIATIVE RECOGNITION COINCIDENT T CELL PARADIGMS?
J.W.Streilein, Univ. of Tex. Health Science Center, Dallas, Texas 75235

T lymphocytes are obsessed with cell surface molecules encoded by genes of the MHC, particularly Class I (K/D) gene products. In one paradigm,T cells recognize and are activated by allodisparate Class I antigens (allogeneic recognition); in another paradigm, T lymphocytes recognize syngeneic Class I determinants only when modified by viral antigens or when derivatized by haptens (associative recognition). Whether these paradigms are coincident, or even overlapping, is unresolved.

We have studied neonatal tolerance among the H-$2K^b$ parent strain and several of its Class I mutants as a means of analyzing the fine discrimination potential of T cell recognition. Neonatal C57BL/6 animals were rendered tolerant of K^b mutant antigens; bml,bm3,bm5 or bm8. Similar panels of mutant mice were rendered tolerant neonatally of the parent K^b antigen. When fully tolerant,mature animals were challenged with third party grafts bearing related K^b alloantigenic disparities,they proved to be highly discriminant. K^b animals tolerant of bml or bm3 always rejected third party brafts bearing bm3 and bml antigens, respectively. Similarly, the majority of K^b animals tolerant of bm5 or bm8 (and vice versa) were able to distinguish accurately the tolerated antigen from third party antigens, rejecting grafts bearing the latter.

Since associative recognition involving these antigens has been found to be at least partially degenerate (Zinkernagel and Klein, Immunogen. 4:581,1977; Forman and Klein,Immunogen. 4:183,1977),and since neonatally tolerant animals nonetheless remain highly discriminant of these same antigenic variations, it is tempting to conclude that associative and allogeneic recognition address different aspects of T cell function.

410 STRUCTURAL STUDIES ON A MURINE HISTOCOMPATIBILITY ANTIGEN SPECIFIED BY THE H-2L LOCUS, Thomas J. Kindt, John E. Coligan, Rod Nairn, Stanley G. Nathenson, David Sachs and Ted Hansen, National Institutes of Health, Bethesda, MD 20205 and Albert Einstein College of Medicine, Bronx, NY

Evidence has been obtained by cellular and immunochemical studies that certain murine H-2 haplotypes possess a third locus (in addition to K and D) that encodes a classical transplantation antigen. This locus is designated H-2L. Genetic studies have been unable to separate the H-2D and H-2L loci by recombination indicating that they are very tightly linked. In the present study molecules were isolated using antisera directed against the H-$2L^d$ MHC gene product. The molecule was isolated by immunoprecipitation from detergent lysates of H-2D tumor cells labeled by growth in culture with radioactive amino acids. The 44,000 m wt H chain of the molecule was isolated and six major fragments were prepared by cyanogen bromide digestion. Because of the high degree of amino acid sequence homology with other major transplantation antigens, it was possible to align these fragments to other K and D gene products. Sequence comparisons indicated that approximately 80% of the residues in the H-2L molecule are identical to those found for H-$2D^d$, H-$2K^b$, and H-$2K^d$. It was furthermore found that the differences between the L^d and K^b and D^d molecules are distributed throughout the amino acid sequence. These data indicate that the H-$2L^d$ gene product is a molecular entity distinct from but homologous to the H-2K and H-2D gene products.

411 SEROLOGICAL AND BIOCHEMICAL IDENTIFICATION OF HYBRID Ia ANTIGENS, W.P. Lafuse, J.F. McCormick and C.S. David, Mayo Clinic, Rochester, MN 55901

Genes within the I region of the H-2 gene complex code for two serologically and biochemically detectable products mapping in the I-A and I-E subregion. Recent biochemical studies have shown that the I-E subregion product is an hybrid molecule formed by complementation of genes mapping in the I-A (β chain) and I-E subregion (α chain). In this communication we present serological and biochemical evidence that Ia specificities 22 (E^k) and we (E^d) are determinants present on such hybrid Ia molecules. Anti-Ia.22 sera killed approximately 50-60% of the (B10 x B10.D2)F_1 cells up to a titer of 1 in 160 while no lysis was obtained with the two parental cells. This suggests Ia.22 is expressed on the hybrid molecules generated by complementation of A^b with E^d. Similarly, expression of Ia.23 was found in [D2.GDxB10.A(5R)]F_1 while D2.GD and B10.A(5R) lack it. Hence, Ia.23 can be generated by complementation of A^d with E^k. The expression of Ia.22 on (B10xB10.D2)F_1 cells and Ia.23 on [D2.GDxB10.A(5R)]F_1 cells were confirmed by immunoprecipitation and analysis by SDS polyacrylamide gel electrophoresis. Expression of Ia.22 was also found on (B10.SxB10.D2)F_1 suggesting complementation between A^s and E^d and expression of Ia.23 was found on (D2.GDxB10.P)F_1 suggesting complementation between A^d and E^p. Sequential immunoprecipitation studies of (B10xB10.D2)F_1 mice indicate that there are two independent I-E molecules in these mice, one molecule bearing Ia.22 and Ia.7 (A^bE^d) and another molecule bearing Ia.23 and Ia.7 (A^dE^d). These results suggest that Ia.7 is present on the alpha chain coded by the I-E subregion and Ia.22 and Ia.23 are expressed either on the beta chain coded by the I-A subregion or generated by the hybrid molecule. (Supported by NIH grants AI-14764 and CA-24473).

412 DETECTION OF WEAK ALLOANTIGENS ON THE UNFERTILIZED EGG AND PREIMPLANTATION DEVELOPMENTAL STAGES IN THE MOUSE, Susan Heyner and R.D. Hunziker, Philadelphia College of Pharmacy and Science, Philadelphia, PA 19104

Mouse oocytes at the dictyate and metaphase II stages as well as fertilized eggs and cleavage stages have been studied with multispecific alloantisera directed against non-H-2 alloantigens. Immunofluorescent studies with oocytes from appropriate target strains in conjunction with adsorption studies reveal that both specified histocompatibility (H-antigens) and specified lymphocyte differentiation antigens (Ly antigens) can be detected on dictyate and metaphase II oocytes. When fertilized eggs and cleavage stage embryos were examined, differential patterns of reactivity were discovered. Histocompatibility antigens were weakly positive on fertilized eggs, and could not be detected on 2-cell stages, but could be detected by the 8 - 64 cell stage and thereafter. In contrast, Ly antigens could be detected on stages up to the 8-cell stage, but not on morulae or blastocysts. Supported by grants HD 11566 and K04 HD 00223

413 THYMUS DIRECTED T CELL MATURATION AND SELECTION, Stephen M. Hedrick, and James Watson, Department of Microbiology, University of California, Irvine, CA 92717

The differentiation of hematopoetic stem cells to T lymphocytes occurs as a result of influences from the thymus. The processes involved in this differentiation include not only maturation to immunocompetent T cells, but a selection for T cells able to specifically interact with cells bearing H-2 complex encoded determinants expressed in the thymus. This has been shown by studies involving the use of thymus chimeras. H-2 heterozygous (bxd) mice were bred with the homozygous nude (athymic) mutation. These congenitally athymic mice were provided with a fetal thymus graft as a stimulus and environment for T cell maturation. If mice were grafted with an H-2 homozygous thymus from a parental strain donor, host T cells which arose were shown to be restricted to the induction of antibody synthesis in B cells and MΦ bearing the thymic H-2 haplotype. This restriction of T cell interaction could be demonstrated as early as 12 weeks and at least as long as 10 months after thymus transplantation. The finding that the thymus directs the specificity of a T cell population for cellular recognition, correlates with the phenotypic expression of immune response genes. In thymus chimeric mice which are (high responder x low responder)F_1, the origin of the thymus has been shown to determine the expression Ir-collagen gene function. To further probe the nature of thymus directed Ir gene control, current studies are directed toward the examination of a recently discovered variant strain of mice. This strain expresses a low responder H-2^d haplotype by serological analysis, but produces a consistently high antibody response to collagen.

414 ANTIGEN REQUIREMENTS FOR ACTIVATION OF CYTOTOXIC LYMPHOCYTES, Charles G. Orosz, Stuart Macphail and Fritz H. Bach, IRC, University of Wisconsin, Madison, WI, 53706.

Unprimed murine spleen cells do not generate cytotoxicity *in vitro* in response to cytotoxic target (CD) determinants presented by H-2 K different thymocytes. To respond, these cells must also receive the antigenic stimulus presented by H-2 I different spleen cells, although by themselves such cells induce little or no cytotoxicity against H-2 K disparate target cells. Spleen cells primed *in vitro* to H-2 K plus H-2 I antigens and tested 14 days post priming also do not respond to H-2 K different thymocytes. However, these cells can be stimulated to re-express high levels of cytotoxicity (directed at H-2 K disparate target cells) when cultured with H-2 I different stimulator cells. Spleen cells from mice primed *in vivo* to H-2 K plus H-2 I antigens and tested 30 days post priming can be restimulated to express cytotoxicity by H-2 K different thymocytes but not by H-2 I different spleen cells. If both K and I disparate stimulators are present, there is a marked synergistic increase in the generation of cytotoxicity.

Based on these differential responses to H-2 K and H-2I encoded alloantigens, it is apparent that: (1) both *in vivo* and *in vitro* priming lead to the generation of a cell population that behaves differently than non-sensitized cytotoxic precursors; and (2) cytotoxic memory cells from 30-day *in vivo* priming are different from memory cells generated following 14-day *in vitro* priming, since *in vivo* but not *in vitro* priming generates a cytotoxic precursor responsive to H-2 K different thymocytes.

415 MULTIPLE PATHWAYS OF T-T INTERACTION IN THE GENERATION OF CYTOTOXIC T LYMPHOCYTES, R.B. Corley and K.A. Switzer, Duke University, Durham, N.C. 27710

Helper T (Th) cells participate in the generation of cytotoxic T lymphocytes (CTL) to alloantigens. However, little is known about the mechanisms which regulate the interaction of Th cells with CTL precursors (CTL.P). We have investigated the nature of Th-CTL.P interactions using unprimed and primed populations of Th cells with syngeneic and allogeneic CTL.P. The results demonstrate that at least two pathways of T-T interaction can lead to the activation of cytotoxic lymphocytes. The first is an allogeneic effect in which Th cells recognize and respond to alloantigens on CTL.P. The second is the interaction of Th cells with syngeneic CTL.P. The latter interaction can be shown to be restricted by H-2-linked determinants when allogeneic effects against CTL.P are minimized. Restricted interactions are observed only between primed Th cells and CTL.P but not between unprimed Th cells and CTL.P. That MHC-linked gene products influence the ability of Th cells to interact with CTL.P indicates that interpretations of experiments using radiation chimeras, in which the existence of Th cells in the generation of CTL to alloantigens has been questioned and restricted interactions between these cells is not predicted, must be modified.

416 I-A and I-E(C) REGION PRODUCTS AS RESTRICTING ELEMENTS FOR HAPTEN SPECIFIC HELPER T CELLS, Carlos Martinez-A., Antonio Coutinho and Rosa R. Bernabé, Basel Institute for Immunology, Basel, Switzerland.

Most, if not all, T cells recognize products of the major histocompatibility complex (MHC), either as alloantigens or as restricting elements in the responses to non-MHC antigens. Helper T cells can be raised in CBA mice with specificity for syngeneic spleen cells derivatized with each of the three haptens, TNP, FITC and SP. These helper cells are exquisitely specific for the homologous antigens, since they express helper activity derivatized with hapten concentrations 100-fold lower than those used for priming, while being totally unable to recognize the heterologous hapten modifications. Expression of helper activity i.e., induction of target B cells, requires the simultaneous recognition of antigen and I-A or I-E(C) encoded determinants directly on the responding B cell surfaces. The fraction of helper activity restricted to I-E(C) has been in the range of 10 to 25% of the total (I-A and I-E restricted) helper activity recovered from CBA cultures in different experiments. The fact that only a relatively small fraction of the helper activity is I-E(C) restricted can explain why this phenomenon has passed unnoticed thus far. We could only detect it, we feel, because of the high sensitivity of our helper assay. The nature of the I-E(C) encoded restricting elements must be considered in the context of the work by Jones who has shown that the most polymorphic chain of I-E(C) antigens (E_β) is actually coded for at the I-A region. Our results indicate that the presence of *k* at I-E is sufficient for functional reactivity of helper cells, regardless of the E_β chains originating at I-A regions carrying k, s or b alleles.

417 COMPARISON OF T CELL EFFECTOR MOLECULES DERIVED FROM NORMAL CELLS, T CELL HYBRIDOMAS AND LONG TERM CELL LINES, Marc Feldmann, Sirkka Kontiainen, Larry Winger, Mike Cecka, Eric Culbert and Liz Simpson, Department Zoology, University College, London WC1E 6BT
Attempts to characterize factors have been hampered by the lack of sufficient material, and the heterogeneity of what is available. Thus we have attempted two approaches to making large quantities of monoclonal factors, by making T-T hybrids, or normal growing cells in the presence of growth factors. Antigen specific suppressor and helper factors were produced by a variety of cell lines - SF to proteins such as KLH, synthetic polypeptides such as GAT, haptens such as NP have been produced using BW5147 as a fusion partner. Helper cells have been more difficult to produce and so other HAT sensitive cell lines have been tried. In general terms the factors produced have been analogous to factors produced by normal cells - in binding characteristics, function, presence of Ia and reaction with antifactor antisera. The quantities released have permitted a biosynthetic labelling approach.
Long term cell lines, both mouse and human have also been found to produce factors which resemble those from normal cells in functional respects antigen specificity, and immune activity. Attempts to characterize factors from cell lines are still in progress, as well as testing whether these helper cells are MHC restricted. A model of T cell factor structure, correlated from these approaches will be presented.

418 T CELL HYBRIDOMAS PRODUCING ANTIGEN SPECIFIC SUPPRESSOR FACTORS, Sirkka Kontiainen, Marc Feldmann, Mike Cecka, Ian Todd and Elizabeth Simpson, Department of Bacteriology and Immunology, University of Helsinki, Helsinki, Finland, ICRF Tumour Immunology Unit, Zoology Department, University College London, Gower Street, London WCIE 6BT, Clinical Research Center Harrow, Middlesex, London.

In vitro induced keyhole limpet haemocyanin (KLH) or hapten NP specific suppressor cells were fuzed with AKR BW 5147 thymoma using polyethyleneglycoll. Clones with suppressive supernatants were further analyzed. The cell surface phenotype was assayed using anti H-2, anti-Ly, anti I-J and anti mouse Ig antisera, and the number of chromosomes estimated. Supernatants from these clones were tested for antigen specific suppression. Suppressor factors secreted by hybrids resembled conventional antigen specific suppressor factors in having a similar MW (about 50-70 000), carrying serologically definable constant and idiotypic region determinant as well as antigen combining site and I-J coded determinants. Biosynthetic labelling revealed a complex pattern, presumably indicative of two chains.
NP specific hybrids were of interest as in this system, it is possible to characterize the material with respect to idiotype. Fusion products of BW 5147 and B10 NP specific suppressor cells yielded a set of clones (possibly identical) which release factors with $I\text{-}J^k$ (derived from BW), NP specificity, and on prelimainary analysis with the V_H NP^b idiotype, indicating B10 origin of the NP combining site. These results suggest that at least two sets of genes contribute to fact structure - in this case AKR & B10 derived. The simplest model is two chains, one with I-J, other containing the combining site.

419 ERYTHROCYTE ANTIGEN H-2.7(G): EXPRESSION IN PLASMA, MAPPING TO S REGION AND LOCATION ON C4, S. Yokota, K.W. Beisel and C.S. David, Mayo Clinic, Rochester, MN 55901
H-2.7 specificity was defined on the basis of hemagglutination reaction and by in vivo absorption. The positive haplotypes by direct hemagglutination were f, j, p and s. $H\text{-}2^k$ haplotype was found positive only by in vivo absorption. Several recombinants mapped the specificity to the left of the H-2D region, while A.TFR-1 mapped it to the right of the S region into a new region, H-2G. Recent studies have shown that the positive hemagglutination reaction of A.TFR-1 with anti-H-2.7 was due to a cross-reactivity at the H-2D region. New restricted anti-H-2.7 do not show hemagglutination with this strain, and hence, maps H-2.7 between the I and D into the S region. Plasma from the H-2.7 positive strains inhibit hemagglutination with anti-H-2.7. Plasma of $H\text{-}2^k$ haplotype showed a much decreased inhibition. Since $H\text{-}2^k$ haplotype is Ss^l expressing a reduced amount of C4, a possible correlation between expression of H-2.7 in plasma and C4 level was suggested. An F_1 ($H\text{-}2^b \times H\text{-}2^k$) (haplotype $H\text{-}2^b$ is Ss^h and H-2.7 negative) which contains an intermediate level of C4 in plasma inhibited anti-H-2.7, suggesting that $H\text{-}2^k$ haplotype does express the structural gene coding for H-2.7 antigen, but requires the presence of the Ss^h gene for its expression in plasma. The F_1 animals gave a weak direct hemagglutination, suggesting that expression of H-2.7 on the erythrocyte depended on the level of C4 in plasma. Anti-Ss and anti-human C4 were able to remove the inhibiting ability of plasma from H-2.7 positive strains, suggesting that H-2.7 antigen may be a determinant on C4. Recently, Ferreira et al. (1980) have positively identified H-2.7 specificity as an antigenic determinant on the γ_1 chain of C4. (Supported by NIH grants AI-14764 and CA-24473).

420 DISTINCTION BETWEEN MYELOID AND B LYMPHOCYTE PROGENITORS. C.J. Paige, L.A. Shinefeld, and V.L. Sato. Sloan-Kettering Institute, Rye, NY and Harvard University, Cambridge MA
This study was undertaken to examine the relationship between *in vivo* spleen colony forming units (CFU-s) and early precursors of B lymphocytes. Normal, chromosomally marked, CBA/H-T6T6 cells were transferred into immunodeficient CBA/N mice. The emergence of donor B cells was monitored by means of a cloning assay (in which CBA/N cells are inactive) and by karyotypic analysis of lymphoid, myeloid, and stem cell metaphases. Injection of 12-13 day fetal liver or adult bone marrow resulted in the rapid generation of B cells (< 1 week) whereas injection of 10 day yolk sac, containing the same number of CFU-s, failed to generate B cells until after 8 weeks. A similar lack of correlation between CFU-s and B cell reconstitution was also found using fetal liver of different gestation ages. Among the possible explanations for these results are: 1)CFU-s alter their ability to generate B cells during ontogeny; 2)under the conditions employed, CFU-s compete poorly with other progenitors in the generation of B cells; or 3)CFU-s are not progenitors of B lymphocytes. To further distinguish among potential B cell precursors we have used monoclonal antisera which are cytotoxic for hematopoietic cells. One rat anti-mouse hybridoma, 19B5, reacts with the pre-B cell line 70Z/3 but not with CFU-s. Exposure of fetal liver or adult bone marrow to 19B5 + complement, prior to use in the reconstitution assay, led to a 60-99% reduction in B cell levels in the recipient mice despite the presence of normal numbers of CFU-s in the injected cell populations. These results suggest that B lymphocytes, from mid-gestation through adult life, are derived from progenitor cells not dependent on CFU-s and that the latter may be limited to other lines of differentiation.

421 SEQUENTIAL EXPRESSION OF B LYMPHOCYTE SURFACE ANTIGENS *IN VITRO*. Joan Abbott and Kin Ngiam, Sloan-Kettering Cancer Center, New York, NY 10021
Bone marrow cells from 7-8 day mice were cultured *in vitro* and tested at 24 hour intervals for their competence to express B cell surface antigens in response to a three hour induction with *E. coli* lipopolysaccharide (LPS). LyB-2 can be induced on day 1, IgM can be induced only after 24 hours, Ia after 48 hours and IgD after 72 hours in culture. This sequence mimics the appearance of B cell antigens during ontogeny. Induction of antigen was demonstrated by the cytotoxicity assay, quantitative absorption and the protein-A sheep red blood cell rosetting assay. When DNA synthesis is blocked from 0-24 hours with hydroxyurea (HU) or cytosine arabinoside (Ara-c) all markers are induced simultaneously by LPS at 24 hours. This suggests that the appearance of cells inducible for these markers does not require progression through a complete cell cycle. When cells bearing one marker are removed by mass cytolysis, there is a coordinate loss of cells bearing other induced B cell markers demonstrating that the induced surface antigens appear together on the same cells. Cells incubated with both HU and LPS from 0 time and assayed for antigen expression at 8, 11, 14, 17 and 19 hours, show no induction until 14 hours when both IgM and Ia appear. IgD expression does not occur until 19 hours. It appears that although slowing down the cell cycle causes the premature appearance of cells inducible for Ia and IgD, there is still some regulation of the sequence in which they appear within the cell cycle. Since both HU and Ara-c are known to cause cells to accumulate at the G1/S interphase, we are currently investigating the possible role of G-1 in the regulation of sequential induction of surface antigens.

Role of Protein Phosphorylation in Control of Cellular Development and Division

422 MITOGEN-STIMULATED AND cAMP-DEPENDENT PROTEIN PHOSPHORYLATION IN 3T3 CELLS, Marit Nilsen-Hamilton and Richard T. Hamilton, The Salk Institute, Box 85800, San Diego,CA
Fibroblast growth factor (FGF) and serum stimulate rapid alterations in the phosphorylation of a number of proteins in quiescent ^{32}P-labelled 3T3 cells. The increase in phosphorylation occurs rapidly and is readily detectable after five minutes of incubation with the mitogen. The increase in incorporation of ^{32}P into a 33,000 dalton protein, identified as the S6 protein of the 40S ribosomal subunit is greater than the increase in incorporation of ^{32}P into most other cell proteins. FGF and serum do not stimulate phosphorylation in ^{32}P labelled SV40-transformed 3T3 (SV3T3) cells or in growing 3T3 cells. Both growing and quiescent 3T3 cells possess cAMP-dependent protein kinases that phosphorylate endogenous protein substrates. Incubation of microsomal fractions from 3T3 cells with ^{32}ATP in the presence and absence of cAMP, produces the same alterations in phosphorylation pattern as found in microsomes isolated from quiescent ^{32}P-labelled 3T3 cells treated with FGF or serum. The cytoplasmic fractions of both 3T3 and SV3T3 cells possess types I and II histone kinase activities yet cAMP only stimulates the phosphorylation of the S6 protein in microsomes from 3T3 cells. It can be demonstrated that the S6 protein is present in microsomes from SV3T3 cells and can be phosphorylated by beef heart cAMP-dependent protein kinase. Our results show that the effect of FGF and serum on the incorporation of ^{32}P into proteins in quiescent 3T3 cells can be mimicked *in vitro* by cAMP. Comparison of histone kinase and endogenous protein kinase activities in 3T3 and SV3T3 cells show that there is not a direct correlation between the amount of histone kinase activity and the ability of endogenous protein kinases to phosphorylate the S6 ribosomal protein in a cAMP-dependent manner.

423 POLYOMA MUTANTS WITH ALTERED MIDDLE T-ANTIGENS, David I. Dorsky and Thomas L. Benjamin, Department of Pathology, Harvard Medical School, Boston, MA 02115

Mutants of polyoma have been isolated which have altered middle T-antigens. Two of the mutants appear to have unchanged large (100K) T-antigen. A third mutant seems to specify an altered and defective large T-antigen as well as an altered middle T. In all three mutants, the size of small (22K) T-antigen appears unaltered, suggesting that the mutations occur distal to the small T-antigen terminator. One of the mutants producing unaltered large and small T-antigens also specifies middle T-antigens with increased thermolability at 39° relative to wild-type. The effects of these mutations on transformation and middle T-antigen associated protein kinase will be reported.

424 MEMBRANE PROTEIN PHOSPHORYLATION FOLLOWING ROUS SARCOMA VIRUS TRANSFORMATION OF CHICK EMBRYO FIBROBLASTS, Daniel P. Witt, Donald J. Brown and Julius A. Gordon, Department of Pathology, University of Colorado School of Medicine, Denver, Colorado 80262

We have studied protein phosphorylation in the membranes of normal and Rous sarcoma virus transformed chick embryo fibroblasts by the methodologies of cell fractionation and two dimensional gel electrophoresis. Following a 2 hour incubation with $^{32}P_i$, about 6-8 major phosphoproteins can be identified as membrane associated in the normal cell. The phosphorylation of a pair of these proteins was found to be dramatically reduced following transformation. These two proteins have molecular weights of about 55-60K daltons, and electrofocus in the O'Farrell system at a pH around 5.1. Studies with a temperature-sensitive mutant of the Rous sarcoma virus (ts NY68) indicate that the time course of the alterations in phosphorylation correlates well with other parameters of transformation. For example, when the temperature-sensitive transformed chicken embryo fibroblast is shifted from the permissive (35°) to the nonpermissive temperature (41°), an increase in the phosphorylation in these proteins is seen within several hours.

Two hour pulse and 4 hour chase studies in intact cells have been done and suggest that the turnover of the phosphate group on both proteins is slow in normal cells. This is supported by the finding that phosphorylation in isolated membranes utilizing endogenous protein kinases and γ-^{32}P-ATP resulted in no detectable labeling of either protein with only a 2 minute incubation. We do not yet know whether the changes in phosphorylation seen in transformed cells result from an activation of a specific phosphatase, subtle changes in the 60K membrane proteins themselves, changes in membrane topology, or other factors.

425 Guanine nucleotide binding activity as an assay for the src protein of Kirsten or Harvey sarcoma virus by E. Scolnick, T. Shih, O. Lowy, E. Chang, G. Hager and C.Wei

We have identified a 21,000 dalton protein coded for by KI-MSV or Ha-MSV, and shown that the p21 is required for the maintenance of transformation by either virus. When extracts of KI-MSV or Ha-MSV transformed cells are incubated with [^{3}H]-GDP, pmol quantities of guanine nucleotide can be immune precipitated with antisera that contain antibodies to p21. Binding of [^{3}H]-GDP is thermolabile both in vivo and in vitro in extracts of cells transformed by Ts371 KI-MSV, a mutant ts for the maintenance of transformation. In addition the immune precipitability of the ^{35}S-methionine labelled p21 in extracts of ts371 KI-MSV transformed cells can be preserved if such extracts are incubated with added GDP or GTP prior to heating. The results suggest an interaction between p21 and certain guanine nucleotides and experiments are in progress to identify the metabolic pathway that p21 and GDP or GTP participate in. Using molecularly cloned infectious Ha-MSV DNA, we have obtained transforming subgenomic DNA fragments of Ha-MSV and created deletion mutants in different regions of the genome. A region critical for transformation maps in the 5' thirty percent of the Ha-MSV genome. Each transformant synthesizes p21 and contains the ^{3}H GDP binding activity. The results constitute the first genetic proof of the role of a putative src protein in the maintenance of transformation by a mammalian sarcoma virus.

426 RELATIONSHIP OF S6 PHOSPHORYLATION TO PROTEIN SYNTHESIS AND cAMP LEVELS DURING G_1, George Thomas, Julian Gordon, Luis Jimenez de Asua, Friedrich Miescher-Institut, P.O.Box 273, CH-4002 Basel, Switzerland.
Stimulation of resting 3T3 cells to proliferate by serum, led to a rapid multiple phosphorylation of ribosomal protein S6 within minutes. The absolute level of phosphorylation, measured by its electrophoretic shift by 2-D PAGE, revealed 5 unique derivatives representing increasingly phosphorylated forms. By 1 hour post-induction nearly all of S6 had shifted to the most highly phosphorylated form. The initial phosphorylation event preceded the activation of protein synthesis. Furthermore, inhibition of protein synthesis by cycloheximide did not block the serum-induced increase in S6 phosphorylation. However, methylxanthines inhibited S6 phosphorylation and protein synthesis. Indeed, the effect could be titrated, such that at high concentrations, S6 phosphorylation and activation of protein synthesis were completely blocked. These results suggest that stimulation of S6 phosphorylation is a necessary prerequisite for activation of protein synthesis and not a consequence of it. In addition, previous results along with those presented here were consistent with a negative correlation between cAMP levels and S6 phosphorylation and protein synthesis. However, serum stimulation of cells in the presence of PGE, raised cAMP levels 10-fold with no effect on S6 phosphorylation or protein synthesis.

427 CYCLIC AMP MEDIATED PROTEIN PHOSPHYLATION AND THE REGULATION OF CELL MORPHOLOGY, Arthur H. Lockwood and George S. Bloom, University of North Carolina at Chapel Hill.
Elevation of intracellular cyclic AMP causes many oncogenically transformed fibroblasts cell lines to regain normal morphology and growth control. We have found that such "reverse transformation" of CHO cells by dibutyryl cAMP is accompanied by a major redistribution of myosin into stress fibers together with an increase in the mass and length of microtubules. Since the major known pathway for cAMP action is the activation of cAMP dependent protein kinases, we have examined the patterns of in vivo protein phosphylation in cAMP treated CHO cells. High resolution gel electrophoresis demonstrates only two striking changes that correlate with morphological reversion by cAMP: a protein of 55,000 daltons becomes phosphorylated while a protein of 20,000 daltons is dephosphorylated. Subcellular fractionation and in vitro studies have been carried out to identify these unique phosphoproteins and to determine their possible functions in mediating morphological reversion and growth control. Related studies that demonstrate an effect of cAMP mediated phosphorylation of tubulin assembly proteins on the stability and assembly of microtubules from brain and CHO cells will be described.

428 GROWTH CONTROL IN ROUS SARCOMA VIRUS-TRANSFORMED CHICK EMBRYO FIBROBLASTS. Robert R. Friis, Andy Ziemiecki, and Heinz Bauer, Institut für Virologie, Fachbereich Humanmedizin, 6300 Giessen, W.-Germany.
Growth control has been studied using cells infected with phenotypically different mutants of Rous sarcoma virus in temperature shift experiments. Quiescent cells, obtained by growing mutant-infected cells at the nonpermissive temperature for 48 hrs in the absence of serum, were followed after downshift using increased uptake of uridine, phosphate, and rubidium, and thymidine incorporation as markers for intracellular events. Appearance of the $pp60^{src}$ associated protein kinase, and the state of phosphorylation of the $pp60^{src}$ were followed in parallel. Influence of nutritionally-deficient medium on growth induction was examined. Notable among the observations were the following: 1) the kinetics and qualitative sequence of events occuring upon downshift of mutant-infected cells were indistinguishable from those observed with normal cells which received serum added-back after a similar period of deprivation, 2) $pp60^{src}$-associated protein kinase, and fully phosphorylated $pp60^{src}$ appear rapidly after downshift with most mutants, however, two mutants fail to show these paramters, but do show growth induction, 3) experiments in which cells were induced to grow under conditions of deficient medium (low pH, glucose-free, or phenylalanine-free) show that transformed cells, but not normal cells, undergo growth suicide.

429 SIMILARITIES BETWEEN A cAMP-INDEPENDENT PROTEIN KINASE PHOSPHORYLATING PYRUVATE KINASE TYPE M_2 AND $pp60^{src}$-ASSOCIATED PROTEIN KINASE OF ROUS SARCOMA VIRUS.

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Proliferating cells of granulation tissue and different tumors including Rous sarcoma virus (RSV) induced tumors contain high amounts of pyruvate kinase M_2 type (M_2-PK) as shown by immunohistochemical investigations. The distinct kinetics of M_2-PK reported for RSV transformed chicken embryonic cells (CEC) are in part caused by changes in the M_2-PK interconverting system(1). The degree of phosphorylation of M_2-PK after in vivo labelling with [^{32}P]orthophosphate is higher in CEC after transformation by RSV compared to Rous associated virus infected CEC. Characterizing a cAMP-independent protein kinase phosphorylating M_2-PK (2) from RSV induced tumors in chicken we found functional similarities to the $pp60^{src}$-associated protein kinase(3). Although both protein kinases are tested with different substrates (immunoglobuline or M_2-PK) both display a broad nucleotide specificity and are inhibited by phospho-ribosylepyrophosphate, fructose1,6-diphosphate and P^1,P^5-di(adenosine-5')pentaphosphate. Both are copurified by different purification methods with M_2-PK.

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3. Collett, M.S. and Erikson, R.L. (1978) Proc. Natl. Acad. Sci. USA 74, 2021-2024

430 CLOSE ASSOCIATION BETWEEN $pp60^{src}$-ASSOCIATED PROTEIN KINASE AND PYRUVATE KINASE TYPE M_2,

Helga Rübsamen[1], Peter Presek[2], Hartmut Glossmann[2], Robert Friis[3], Heinz Bauer[3], Wilhelm Schoner[4], and Erich Eigenbrodt[4], Institute of Virology, University of Köln[1], Institutes of Pharmacology[2], Virology[3], and Endocrinology[4], University of Gießen, W.-Germany

We have purified the $pp60^{src}$ associated protein kinase as defined by Collett et al. (1) using 3 different affinity chromatographic systems, blue sepharose, casein-sepharose and a 33000 d inhibitor protein of the glycolytic enzyme pyruvate kinase (2) coupled to sepharose 4B. In all cases, pyruvate kinase type M_2 (M_2PK) was found to copurify with the $pp60^{src}$-associated kinase activity, sometimes, however, enzymatically inactive. Upon extraction from the cells under mild conditions (isotonicity, 0.05% Triton X-100) followed by gel filtration, the $pp60^{src}$ associated kinase activity migrated with several apparent molecular weights, >500000, 300000, 160000 and < 100000 d. The 300000 and 160000 d peaks also contained M_2PK. The comigration of the 2 enzymes observed in all chromatographic systems, even at high levels of purification of the $pp60^{src}$ kinase activity is considered to strongly suggest the formation of a specific complex. This finding is specially interesting in view of the fact that M_2PK is phosphorylated by a cellular enzyme of 60000 d and similar enzymatic characteristics as the kinase activity associated with the protein coded for by the transforming gene of Rous sarcoma virus, $pp60^{src}$ (Presek et al., this conference).

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431 PHOSPHORYLATION OF POLYOMA T ANTIGENS,

Brian S. Schaffhausen and Thomas L. Benjamin, Department of Pathology, Harvard Medical School, Boston, Mass., 02115.

Phosphorylation of polyoma virus T antigens has been examined both in vivo and in vitro. Labeling infected cells with ^{32}P-orthophosphate principally labels the 100K 'large' T antigen. The 56K plasma membrane-associated 'middle' T antigen is also phosphorylated but to a lesser extent. The in vivo phosphorylations of the T antigens of transformation-defective hr-t mutants are similar to those of wild type virus. Hr-t mutants show normal phosphorylation of the 100K T antigen. The altered 56K protein induced by the hr-t mutant NG-59 and the truncated 50K middle T antigen of hr-t mutant SD-15 are also phosphorylated normally. Addition of dibutyryl cyclic AMP to the medium does not affect T antigen labeling.

Hr-t mutants are defective in a protein kinase activity assayed in vitro by adding γ-^{32}P-ATP to T antigen immunoprecipitates. In the case of wild type virus, the 56K protein is the major phosphate acceptor in the in vitro reaction, with a lower level of phosphorylation seen in the 100K band. A wild type strain with a deletion that affects the 100K and 56K bands shows a kinase activity associated with the truncated T antigens. Hr-t mutants NG-59 and SD-15 show no labeling of the altered 56K or 50K species, but do show phosphorylation of their large T antigens. Co-infection with wild type and hr-t mutant viruses or mixing of wild type- and hr-t-infected cell extracts prior to immunoprecipitation does not lead to the in vitro phosphorylation of the hr-t mutant T antigens. Ts-a mutants are normal with respect to the middle T antigen-associated kinase. Transformed cells that lack the 100K T antigen are also normal for in vitro phosphorylation of 56K T antigens.

Cell Surface Receptors

432 HETEROGENEITY OF BINDING AFFINITIES FOR EGF RECEPTORS ON HUMAN AND MURINE CELLS
Kenneth K. Iwata, Robert E. Williams and C. Fred Fox, University of California, Los Angeles, CA 90024
Several reports in the literature describe curvilinear Scathard plots of EGF (epidermal growth factor) binding to mouse and human cells. The significance of this curvilinearity has yet to be resolved. Heterogeneity of EGF binding affinities may be ascribed to 1) negative cooperativity, 2) heterogeneous receptors, 3) increasing rates of down regulation at higher concentrations of EGF thereby reducing the Bound/Free ratio, 4) the origin or developmental state of the cell including the possibility of heterogeneity in the cell population. We have found this heterogeneity of binding affinities present in numerous human and mouse clonal cell lines as well as in cell strains, so that this is not an isolated phenomenon.The heterogenous binding affinities were also observed in membrane preparations derived from human and murine cells showing that it is not a product of EGF induced events in actively metabolizing cells (e.g. varying receptor levels due to down regulation). Dissociation experiments on both membrane preparations and intact cells in the presence of a large excess of unlabeled EGF resulted in two distinct dissociations rates indicative of the presence of at least two specific EGF binding components, as opposed to negative cooperativity for EGF binding.

433 Mechanism of action of epidermal growth factor in bovine granulosa cells.
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Bovine granulosa cells exposed to ^{125}I-labeled epidermal growth factor (^{125}I-EGF) are capable of binding, internalizing, and degrading it within the cells in the lysosomal system. Our results demonstrate that it is possible to inhibit the lysosomal degradation of ^{125}I-labeled EGF without altering the proliferative response of cultured granulosa cells to that mitogen. At a concentration of 80 ug/ml, both leupeptin and anitpain inhibited the degradation of ^{125}I-EGF by at least 90%. When the initiation of DNA synthesis was studied, it was observed that cultures exposed to either saturating or subsaturating concentrations of EGF in the presence of proteases inhibitors exhibited an increase in DNA synthesis which was 70 to 80% that of cultures exposed to the mitogen alone. Moreover, addition of either leupeptin or antipain did not diminish the rate of growth of cultures induced by EGF. In the presence of leupeptin, the ^{125}I-EGF was found to accumulate within the granulosa cells. The ^{125}I-EGF which was found within the cells was precipitable with antiserum against EGF and co-migrated on isoelectric focusing gel with intact ^{125}I-EGF. This suggests that the process of mitogen degradation is not related to the effects of the mitogen on cell proliferation. In our opinion, therefore, the physiological function of the degradation of EGF by the lysosomal system is merely to destroy the mitogen, so that each molecule can act only once. Granulosa cells treated with the lysosomal inhibitor chloroquine showed translocation of about 15% of the accumulated ^{125}I-EGF into the nucleus, where it interacts with non-histone structural proteins. This suggests that a certain percentage of the cell-surface-bound EGF is delivered into the nucleus, possibly by means of fusion with endocytotic vesicles, and that, due to a rapid lysosomal degradation in untreated cells, this pathway, although it does exist, is slightly or not at all detectable in untreated cells because of high background.

434 PRIMARY AMINES DO NOT PREVENT DOWN REGULATION OF EGF RECEPTORS,
C. King, L. Hernaez, P. Cuatrecasas, Wellcome Research Laboratories, Research Triangle Park, NC 27709
The initial interaction of EGF with human fibroblasts involves binding to specific receptors and subsequent internalization and processing of the hormone-receptor complex. Fluorescent EGF analogues suggest that internalization may be preceeded by receptor aggregation which can be prevented by a variety of primary amines. Rapid loss of EGF binding capacity (80-90%) occurs after an initial binding maximum (1 hr) and is essentially complete within 3-4 hours. In the presence of the lysomotrophic agents, methylamine (MA, 5-20 mM) or chloroquine (10-100 uM), down regulation of apparent surface binding does not occur but is replaced by a 2 to 3-fold stimulation. Both the degradation of ^{125}I-EGF and its dissociation are protected by amines, suggesting an inhibition of the degradative processes associated with down regulation. In order to determine whether the increase in apparent EGF binding capacity was caused by the insertion of newly synthesized receptors, experiments with the protein synthesis inhibitor cycloheximide were performed. These results strongly suggest that the EGF receptor is continuously recycled in the basal state in a manner which requires de novo protein synthesis, and that the presence of EGF stimulates normal receptor degradation 3 to 5-fold. Upon preincubation with unlabeled hormone, a 50-75% loss of surface binding capacity occurs even in the presence of 10 mM MA. These receptors are insensitive to mild trypsinization and can be recovered in heterogeneous populations of dense membrane vesiles on equilibrium sucrose density gradients. Thus, receptors which are normally recycled may become "trapped" intracellularly by amines, resulting in increased apparent ^{125}I-EGF binding.

435 MICROTEST ASSAYS FOR BINDING OF RADIOLABELED GROWTH FACTORS AND STIMULATION OF DNA SYNTHESIS,
Peter L. Salk and Jeffrey A. Loeb, The Salk Institute for Biological Studies, San Diego, CA 92138

Microtest assays have been developed for measuring the binding of ^{125}I-labeled mouse epidermal growth factor (EGF) to tissue culture grown cells and the stimulation of DNA synthesis in cultured cells by EGF. The binding assay is performed using confluent cells grown in 96-well microtest plates. After rinsing in serum-free medium (using an 8 or 12 prong manifold) the cells are incubated with ^{125}I-EGF, with and without an excess of unlabeled EGF. After incubation the plates are rinsed and the contents of the wells dissolved in a lysing solution, which is then transfered by cotton swab to small test tubes for gamma counting. Using 80 pg of ^{125}I-EGF (containing 2800 cpm) per well, maximum binding of the growth factor to BALB/3T3 cells was observed at 3 hours incubation at 37°C, with approximately 10% of the added label specifically bound. Stimulation of DNA synthesis was measured 18 hours after the addition of EGF to quiescent BALB/3T3 cells (high density cultures grown in 10% serum or low density cultures grown for 24 hours in 0.1% serum), using similar rinsing and transfer techniques. Maximal stimulation of ^{3}H-thymidine incorporation (500% of control) was observed at an EGF concentration of 12.5 ng/ml in the low density assay and 25 ng/ml in the high density assay. BALB/3T3 sublines which grow to high saturation densities bound ^{125}I-EGF but were not stimulated by EGF in either high or low density assays. Adequate space between the lid and the microtest plate was necessary to promote uniform growth of cells in the outermost wells. These microtest assays permit convenient handling of multiple samples with a minimum expenditure of reagents and should prove useful in studies of growth factors and their antagonists.

436 TPA ENHANCES DNA SYNTHESIS BY TRANSIENTLY REDUCING EGF RECEPTOR AFFINITY AND EGF DEGRADATION,
Bruce E. Magun, Lynn M. Matrisian, and George T. Bowden, Departments of Anatomy and Radiology, Arizona Health Sciences Center, Univ. Arizona, Tucson, AZ

We examined the effect of the tumor promoter 12-O-tetradecanoyl-phorbol-13-acetate (TPA) on the binding of ^{125}I-epidermal growth factor (EGF) to Rat-1 fibroblasts. Scatchard analysis of ^{125}I-EGF binding demonstrated a decrease in receptor number (20-30%) and affinity in the presence of TPA compared to controls. The control curvilinear Scatchard plot can be explained by the presence of two classes of receptors with different affinities. TPA eliminated binding only to the high affinity receptors. The initial TPA-induced decrease in affinity was overcome by 4h, by which time the curvilinear nature of the Scatchard plot was restored. TPA did not alter the rate at which bound EGF was degraded to its major metabolite, monoiodotyrosine. However, TPA and other phorbol esters reduced the amount of EGF that was bound and degraded over a 12h period when the EGF concentration was low (0.2 ng/ml). Under these conditions, inclusion of TPA resulted in 50% more EGF in the medium compared to controls. The synergistic effect of TPA and other promoting phorbol esters on DNA synthesis occurred only at low EGF concentrations, under conditions in which the phorbol ester spared EGF from degradation. We suggest that the ability of TPA to induce DNA synthesis synergistically with EGF relies on the ability of TPA to decrease EGF receptor affinity only temporarily, thus reducing EGF binding and subsequent degradation. After receptor affinity has been restored, higher amounts of EGF would be present to induce a greater population of cells to enter DNA synthesis.
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437 IMMUNOPRECIPITATION OF THE EGF RECEPTOR USING HYBRIDOMA ANTIBODIES.
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Splenic lymphocytes from mice immunized to plasma membrane preparations of the epidermal growth factor (EGF) receptor hyperproducing A431 cells were fused in vitro with murine myeloma cells. Detection of anti A431 antibody-producing "hybridomas" was performed by a direct binding of hybridoma culture supernatants to A431 monolayers, followed by the addition of radiolabelled rabbit anti mouse IgG. Hybridoma cultures positive for Ig to A431 antigens were expanded and the cells were further grown in pristine primed mice to elicit ascites. Fourteen of 18 resulting ascites preparations remained positive for binding to A431, even after the Fc receptors on the A431 cells were blocked by preincubation with nonimmune mouse serum. Attempts to confirm the specificity of these hybridoma antibodies has been done by two methods: cell binding assays; and immunoprecipitation of detergent solubilized membrane preparations from various cells types. Eighty-two percent of the samples tested were positive for binding to human foreskin fibroblasts, which also express the EGF receptor. A majority of the hybridoma antibody samples was found to be functional in immunoprecipitation of the ^{125}I-EGF-receptor complex, and also for significant reduction of the EGF binding potential from nonlabelled A431 membrane preparations. Biochemical characterization of the immunoprecipitated material containing the EGF receptor is in progress.

438 CELL CYCLE AND CHEMICAL TRANSFORMATION EFFECTS ON EPIDERMAL GROWTH FACTOR RECEPTORS,
Robert A. Robinson, Robert J. Ryan and Harold L. Moses, Department of Cell Biology, Mayo Clinic/Foundation, Rochester, Minnesota 55901
Previous studies have indicated that the mouse embryo-derived nontransformed AKR-2B cells have two different G_1 arrest points—a growth factor deficiency arrest point and a low molecular weight nutrient arrest point (Moses et al., J. Cell. Physiol., in press, 1980). Growth factor deficiency arrest is achieved either by use of low serum or by allowing cells to grow to saturation density in 10% serum. Nutrient deficiency arrest is achieved by artificial deprivation of amino acids from the medium or by growing cells in the presence of excess mitogens such as epidermal growth factor (EGF) or 12-O-tetradecanoylphorbol-13-acetate (TPA). A chemically transformed derivative of the AKR-2B cells, called AKR-MCA, arrests in G_1 only at the nutrient arrest point and cannot achieve growth factor deficiency arrest. The AKR-2B cells have approximately 10^5 receptors per cell while the AKR-MCA cells show no detectable receptors. The present study was designed to determine whether lack of detectable EGF receptors in the AKR-MCA cells was influenced by the alteration in cell cycle arrest. AKR-2B cells arrested at the nutrient deficiency arrest point by amino acid deprivation or by maintenance in excess TPA showed a 3 to 10 fold reduction in specific ^{125}I-EGF binding relative to growth factor deficiency arrested AKR-2B cells. The ability to bind ^{125}I-EGF was regained following stimulation of DNA synthesis by replenishment of the nutrients. The alteration in cell cycle control in chemically transformed cells could account for at least part of the reduction in EGF receptors observed in these and other chemically transformed cells (Todaro et al., Nature 264:26, 1976; Hollenberg et al., Cancer Res. 39:4166. 1979).

439 PROTEOLYTIC DOMAINS OF THE EGF RECEPTOR OF PLACENTAL MEMBRANES,
Edward O'Keefe, University of North Carolina, Chapel Hill, NC 27514
The placenta membrane receptor for human epidermal growth factor (hEGF) has been studied by limited proteolytic digestion of affinity-labelled purified microsomal membranes, which contain 20 pmol of receptor per mg protein. Solubilization of the receptor with cholate or nonionic detergents does not permit recovery of binding activity as assessed by Sephadex G-75 elution profile or polyethylene glycol assay using ^{125}I-labelled hEGF. Proteolysis of membranes has not been shown to liberate a fragment retaining ability to bind hEGF. Minimal cross-linking of ^{125}I-labelled hEGF by low concentrations of dimethylsuberimidate specifically labels 180,000 and 170,000 dalton bands on autoradiograms of SDS polyacrylamide electrophoretic gels with minimal alteration of Coomassie blue staining patterns. Limited proteolysis of affinity-labelled membranes produces fragments in parallel with loss of binding of approximate M_r of 160,000 and 80,000 daltons and suggests derivation of the 170,000 from the 180,000 dalton band by autolysis. Identical bands are obtained with trypsin, alpha chymotrypsin, pepsin, elastase, and papain, suggesting that domains of the receptor show differing susceptibility to enzymic attack.

440 THE STRUCTURAL AND FUNCTIONAL TOPOGRAPHY OF ERYTHROCYTE SPECTRIN: SITES OF INTERACTION WITH THE MEMBRANE AND OTHER COMPONENTS OF THE CYTOSKELETON.
Jon S. Morrow, David S. Speicher, William K. Knowles, Jennifer Hsu and Vincent T. Marchesi, Yale University School of Medicine, Department of Pathology, New Haven, Ct. 06510.
Spectrin is the major component of the erythrocyte cytoskeleton. The functional unit consists of two large unique polypeptide chains, comprising a dimer of 470,000 daltons. Each of the component chains, referred to as α & β, is composed of multiple trypsin resistant "domains." The position of these domains, ranging in mw from 80,000 to 28,000 daltons, within the primary sequence of the molecule has been determined by two-dimensional peptide mapping. By the use of proteolytic and chemical cleavage techniques, the sites of interaction of spectrin with the other components of the cytoskeleton have been defined. The tetramer of spectrin is formed by interaction with a terminal 80,000 dalton peptide of the α-chain. This site is near the 2.1 or Ankyrin binding site, which is located on the β-chain. The site of 4.1-actin interaction may be similarly determined. The sites of phosphorylation are exclusively on the β-chain at the end opposite the 2.1 binding site. The α & β chains interact with each other at multiple sites, but most strongly near the ends of the chains. Interactions directly with lipid may provide additional stabilization of the erythrocyte membrane. These findings allow a fairly precise model of spectrin's role in the cytoskeleton.

441 ISOLATION OF BPgp70, A FIBROBLAST SURFACE RECEPTOR FOR THE ENVELOPE ANTIGEN (gp70) OF RAUSCHER MURINE LEUKEMIA VIRUS, Beate Landen and C. Fred Fox, Dept. Microbiol. and The Mol. Biol. Inst., UCLA, Los Angeles, CA 90024

The major envelope glycoprotein (gp70) of murine leukemia and sarcoma viruses is expressed on the surface of some cells in normal murine tissues, and has thus been implicated to play a role in normal developmental processes. Extensive polymorphism has been demonstrated in gp70 by fingerprinting after proteolytic digestion. If the receptor for gp70 also constitutes a polymorphic family of polypeptides, the gp70 species and their receptors would be interesting candidates for an intercellular recognition system.

We have purified BPgp70, a binding protein for the gp70 of Rauscher murine leukemia virus (RMuLV) from the culture fluids used for growth of a clonal murine fibroblast line. This protein is not immune-precipitated by antisera directed against RMuLV-gp70 or whole fetal calf serum. BPgp70 blocks the specific binding of ^{125}I-gp70 to its cell surface receptor by interacting with gp70. It also binds specifically to cells expressing RMuLV-gp70, e.g. JLS-V9-RMuLV or NIH 3T3-RMuLV, yielding an estimated dissociation constant of less than 10^{-8} M. Incubation of BALB/c 3T3 cells with the IgG fraction of an antiserum monospecific for BPgp70 inhibits the specific binding of ^{125}I-gp70 to these cells. Supproted by funds from USPHS (NCI-N01-CP91010) and by a Max Kade fellowship to BL.

442 CELL SURFACE BINDING OF EGF IS ASSOCIATED WITH THE HUMAN CHROMOSOME 7 IN HUMAN-MOUSE CELL HYBRIDS, N. Shimizu, M.A. Behzadian, I. Kondo and Y. Shimizu, Dept. of Cell. & Develop. Biology, University of Arizona, Tucson, Arizona 85721.

A mouse L-cell derived line A9, which is deficient in hypoxanthine phosphoribosyltransferase (EC 2.4.2.8; HPRT), was found to be incapable of binding ^{125}I-labeled epidermal growth factor (EGF) to the cell surface. The A9 cells were fused with human diploid fibroblasts, WI-38, possessing EGF-binding ability and human-mouse cell hybrids (TA-series) were isolated after HAT/ouabain selection. Analyses of chromosomes and isozyme markers of 4 selected clones of TA-hybrids indicated that the expression of EGF-binding ability is correlated with the presence of human chromosomes 7 and/or 19. Four subclones were isolated from an EGF-binding positive line, TA-4, and the segregation of EGF-binding was found concordant with the expression of human mitochondrial malate dehydrogenase (MDHM; EC 1.1.1.37), a marker for chromosome 7, and discordant with glucose phosphate isomerase (GPI; EC 5.3.1.9), a marker for chromosome 19. Furthermore, evidence from 27 clones of AUG-hybrids that were produced between A9 and another human fibroblast line, GM1696, carrying an X/7 translocation chromosome indicated that EGF-binding ability segregrates together with MDHM and two X-linked markers, HPRT and glucose 6-phosphate dehydrogenase (G6PD, EC 1.1.1.49). When 2 AUG-clones (C2B5 and C3B4) were processed for "8-azaguanine back-selection" the EGF-binding ability was lost together with the X/7 translocation chromosome and three markers (MDHM, HPRT and G6PD). From these data the expression of EGF-binding ability in the hybrids can be attributed to the genes (EGFS) located in the p22 qter region of human chromosome 7. The EGFS is likely a structural gene for the EGF receptor. Biological and biochemical response of these hybrid cells upon EGF-binding is under investigation. (Supported by Grants from NIH, GM24375 and ACS, JFRA-9).

443 CELL SURFACE RECEPTORS FOR ENDOGENOUS MOUSE TYPE C VIRAL GLYCOPROTEINS AND EPIDERMAL GROWTH FACTOR: TISSUE DISTRIBUTION IN VIVO AND POSSIBLE PARTICIPATION IN SPECIFIC CELL-CELL INTERACTION. U.R. Rapp, Laboratory of Viral Carcinogenesis, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205.

We have described previously the detection and tissue distribution of free cell surface receptors for ecotropic R-MuLV envelope glycoprotein and the growth factor EGF in vivo (De Larco, Rapp, Todaro. Int. J. Cancer 21, 354 (1978). More recently, we have reported the chromosomal map position of the ecotropic viral receptor and its conservation between subspecies of the genus mus (Marshall and Rapp, J. Virol. 29, 501 (1979). This work has shown, for the first time, the presence of multiple, independently segregating cell surface receptor genes specific for different classes of ecotropic type C viral envelope glycoprotein. We have begun to study the tissue distribution of these newly described receptors.

Preliminary studies indicate that some receptors that are recognized by endogenous MuLV may mediate specific cell-cell binding. Thus, fibroblast cells infected with a replication deficient endogenous C3H/MuLV bind thymic lymphoid cells whereas the uninfected cells did not show this activity. Tissue culture derived transforming variants of endogenous type C viruses on the other hand may interact with mitogenic cell surface receptors. For example, we found that recombinant viruses which induce specific tumors in vivo are altered with respect to the cell surface receptor which they use for infection of mouse cells. Since some of these recombinant viruses stimulate cells to grow in agar and to show a decreased serum requirement for growth it may be that their transforming capacity is a consequence of an altered cell surface receptor interaction.

444 PROTEASE-NEXIN: A CELL SURFACE RECEPTOR FOR THROMBIN AND PLASMINOGEN ACTIVATOR,
Joffre B. Baker, David A. Low, Robert L. Simmer, and Dennis Cunningham, Department of Microbiology, College of Medicine, University of California, Irvine, CA 92717
The primary cell surface receptor on normal diploid human foreskin cells for the serine proteases thrombin or plasminogen activator forms a covalent linkage to these proteases and inactivates them. The protease-receptor complexes are then internalized by the cells. This receptor, which we call protease-nexin (PN), has similarities to antithrombin III (AT3), the major inhibitor of thrombin in serum. PN and AT3 link the same proteases and the linkages are stable in the presence of sodium dodecyl sulfate, 2-mercaptoethanol, or chaotropic agents, but are disrupted by hydroxylamine (1M) or by incubation at pH 12. Both PN and AT3 have high affinity heparin-binding sites, and in both cases heparin dramatically accelerates the rate of protease linkage. However, PN is immunologically unrelated to AT3, and is 38K daltons, whereas AT3 is 65K daltons. Although a fraction of PN is tightly associated with the cell surface, the cells release PN into the culture medium. When this released PN links protease molecules the protease-PN complexes can bind tightly to the cells. The heparin-binding site of PN is involved in the cellular association and internalization of protease-PN complexes, because heparin (but not other mucopolysaccharides) at low concentrations (0.2 μg/ml) prevents these processes. The cells appear to synthesize PN because they release it at a constant rate even after 10 days in the absence of serum. PN may play an important role in regulating the levels of serine proteases in the cellular microenvironment.

445 VISUALIZATION OF THROMBIN RECEPTORS USING AN FITC-AMINE LABELED THROMBIN, Darrell H. Carney, University of Texas Medical Branch, Galveston, TX 77550. Highly purified thrombin initiates division of quiescent fibroblasts in serum-free medium. Recent studies have demonstrated that specific receptors for thrombin exist on the surface of these cells and that thrombin must bind to these receptors to initiate cell division (Cell 15,1314,1978). To determine if receptor redistributions following thrombin binding plays a role in thrombin mitogenesis biologically active fluorescent thrombin was prepared. By modifying a procedure for coupling dansyl hydrazine to thrombin (C.D.Yang,J.W.Fenton,II,and R.D.Feinman, In Press) we synthesized a fluorescein isothiocyante (FITC) ethylenediamine compound and coupled it to the thrombin carbohydrate moity. These molecules with about one FITC per thrombin retained full activity as judged by fibrinogen clotting, ability to bind to receptors, and ability to initiate division of mouse embryo (ME) cells. When FITC-amine thrombin (100ng/ml) was incubated at 4° with ME cells, a diffuse fluorescent labeling of the cells was observed by direct fluorescence microscopy. This diffuse pattern appears to reflect specific receptor binding since the pattern is absent or very weak in parallel cultures incubated simultaneously with a 40 fold excess of unlabeled thrombin. When cultures were warmed to 37° (following binding at 4°) the diffuse fluorescence pattern changed to distinct spots. In competition experiments with unlabeled thrombin present, however, distinct spots could also be observed suggesting that some of this apparent aggregation was nonspecific. This unexpected finding emphasizes the need for controls for nonspecific binding in studies aimed at determining receptor-mediated movement of fluorescent peptides.

446 PROTEOLYTIC MODIFICATIONS OF THE EGF RECEPTOR, Peter S. Linsley and C. Fred Fox, Mol. Biol. Inst. and Dept. of Microbiol., UCLA, Los Angeles, CA 90024
Following the binding of Epidermal Growth Factor (EGF) to murine 3T3 cells, the EGF-receptor complex undergoes several proteolytic modifications which may play a role in the ensuing mitogenic response. The initially formed direct linkage complex between EGF and its receptor (Linsley et al, 1979, Nature 278:745-748) on 3T3 cells or human foreskin fibroblasts (HF-15) migrates as an M_r=160,000 species during gradient gel electrophoresis. Isolated membranes from 3T3 and HF-15 cells display additional direct linkage complexes at M_r=145,000 and M_r=115,000; these fragments are often present on mitogenically stimulated intact cells. While intact cells from the human epidermoid carcinoma line, A431, display a direct linkage complex which is distinctly larger than the one observed with 3T3 or HF-15 cells, membrane preparations from all three sources display a similar array of processed products. The M_r=145,000 and 115,000 fragments appear to be the products of two distinct proteases, one of unknown specificity which is activated when cells are scraped from their substratum, and one having a specificity similar to that of trypsin. The EGF binding site on the receptor molecule remains membrane bound after both cleavages. The M_r=160,000 and M_r=145,000 forms of the receptor present in A431 membranes comigrate during gel electrophoresis with the proteins labeled by the rapid EGF stimulated phosphorylation reaction described by Carpenter et al, 1978, Nature 276:409-410. Trypsin treatment of the phosphorylated membranes results in the complete removal of the 32-P incorporated into these bands. A structural model of the EGF receptor has been constructed from this data. Functional implications of this model will be discussed. Supported by grants from ACS and USPHS (AM11148-01 and NIRSA CA 09056 to PSL).

447 MURINE FIBROBLAST CELL SURFACE PROTEINS: IDENTIFICATION AND CHARACTERIZATION WITH MONOCLONAL ANTIBODIES. Edward N. Hughes and J. Thomas August, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

An analysis of the proteins of the murine fibroblast surface is being carried out through the use of hybrid cell lines secreting monoclonal antibodies to individual antigens of complex mixtures. Murine myeloma cells were fused with spleen cells of rats immunized with NIH/3T3 fibroblasts or plasma membranes. Monoclonal antibodies reactive with cell surface antigens were selected by the binding of antibody to intact cells. Proteins identified have apparent molecular weights of 220, 110, 85, 80, 72 and 55 kilodaltons. Among 17 independent antibodies for which a protein target has been assigned, 5 are reactive with the 85,000 dalton protein; 3, with an 80,000; and 2, with a 110,000. The identity of these proteins has been confirmed by 2-D tryptic peptide mapping. The target antigens of M_r-110, 85, 80 and 72 kilodaltons specifically bound to concanavalin A -Sepharose affinity columns. Localization of the proteins to the plasma membrane was verified by precipitation of proteins radiolabeled by lactoperoxidase catalyzed iodination of intact cells. Another noteworthy finding is that one of the antibodies reacted with a major protein induced by heat-shock of cultured fibroblasts. The distribution of these antigens was tested with a panel of murine cell lines. The 220 and 85 kilodalton proteins were present on 18 lines of diverse tissue origin and appear to be structural components common to plasma membranes of many cells. The other proteins have a limited distribution on cells other than the fibroblast and the antigenic determinant of the 80 kilodalton protein appears to be allospecific since it is found on NIH and other related lines but not on BALB/3T3 cells.

448 THE ROLE OF THE INSULIN RECEPTOR IN THE STIMULATION OF GROWTH OF A RAT HEPATOMA CELL LINE BY INSULIN, John Koontz, University of Colorado Health Science Center, Denver, Colorado 80262

In those cell lines where insulin has been reported to be an effective growth peptide there is some question as to whether the hormone is acting through the specific insulin receptor or is binding to and acting through other growth peptide receptors. Of equal importance is the question of whether insulin binding to the cell surface receptor is sufficient stimulus for the subsequent growth response or if internalization and binding to an intracellular receptor such as at the nuclear membrane is a prerequisite for the stimulation of the long term growth response.

A probe of the insulin receptor which is highly specific is the anti-receptor antibody (ARA). This has been verified to interact with the insulin receptor in these cells by its ability to displace iodinated insulin. The purified IgG fraction of this serum is able to displace iodinated hormone and is able to provoke a growth response as measured by (^{3}H)-TdR incorporation and an increase in percent labeled nuclei. The IgG fraction from normal human serum is unable to provoke such a growth response. The ARA•IgG stimulation of growth is dose-dependent and comparable to the displacement of the labelled hormone. Studies are also being conducted to examine the effect of the antibody fragments on displacement of insulin and on the growth response.

These results suggest that insulin interaction with the specific insulin receptor is sufficient to provoke response. Also, the ability of the ARA•IgG to provoke the response implies that internalization of insulin and subsequent binding to intracellular receptors is not required to elicit the long term effects of the hormone.

449 "Carbohydrate Groups of Glycoproteins in a Glycosylation Defective, Low Adherent Mutant Mouse Cell," Blithe, D.L., Pastan, I., Buck, C.A.*, and Warren, L.*, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205. *Wistar Institute, Philadelphia, Pennsylvania 19104.

The protein bound carbohydrates of Balb/c3T3 and of AD6, a mutant with decreased adhesive properties, have been compared. The mutant has a markedly decreased capacity to acetylate glucosamine. The size range of glycopeptides derived from the mutant is sharply restricted as demonstrated by gel filtration on Sephadex G50. While the overall amount of glycosylation is depressed in the mutant cell, a significantly greater proportion of the glycopeptides which are synthesized can bind to Con A relative to those of the parent cell. Analysis by ion exchange chromatography suggests that the mutant, while possessing all of the types of glycopeptide populations found in the parent cell, is relatively deficient in the more negatively charged components.

450 A PROPOSED MECHANISM FOR RECEPTOR-LIGAND INTERACTION IN COATED PITS, John W. Woods and Thomas F. Roth. University of Maryland Baltimore County, Catonsville, MD 21228.

Receptor clustering appears to be a prerequisite to coated vesicle mediated protein sequestration. A possible ligand receptor interaction which could mediate such clustering can be inferred from our studies on phosvitin (PV) binding to chicken oocytes. PV binds to oocyte membranes with an apparent K_a of 3×10^5 M^{-1} as a second-order process. In the absence of free ligand bound PV dissociates slowly from the receptor with first order kinetics. However, in the presence of excess unlabeled ligand PV dissociation is much faster and gives a concave semilog plot indicating some higher order process.

One possible reaction mechanism is: $PV + R = RPV + R = R_2PV$ where all receptors have the same affinity for PV and each PV contains more than one site recognized by the receptor. Thus, free PV initially binds to a single receptor at a rate dependent upon the concentrations of free PV, free receptors, and the affinity of the receptors for PV. However, once PV is bound to a single receptor it can then interact rapidly with other receptors giving rise to clusters of receptors.

A similar mechanism may mediate other receptor ligand interactions where large proteins (such as LDL, IgG, and vitellogenin) are bound to receptors that become clustered and endocytosed in coated pits. In this manner an essentially irreversible receptor-ligand interaction becomes possible in systems where the initial interaction is of much lower affinity. In addition, this mechanism could mediate receptor clustering as a prelude to internalization by coated vesicles.

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451 EXPRESSION OF CHEMOTACTIC RECEPTORS DURING DIFFERENTIATION OF HUMAN PROMYELOCYTIC LEUKEMIA CELLS (HL-60), Itzhak Kahane, James Niedel and Pedro Cuatrecasas, The Wellcome Research Laboratories, Research Triangle Park, NC 27709.

Membrane receptors for the potent chemotactic peptide N-formyl-Nle-Leu-Phe-Nle-Tyr-Lys (formyl peptide) are not present on myeloblasts and immature promyelocytes in HL-60 cultures. The small amount of specific ^{125}I-formyl peptide binding to cells from these cultures is due to the presence of a small (ca. 1%) subpopulation of more mature cells which display the receptor and internalize the peptide in a receptor-mediated process. In response to treatment of the culture with polar compounds (DMF, DMSO) which induce cell differentiation, ^{125}I-formyl peptide binding increased 50- to 100-fold. Using a rhodamine labeled formyl peptide and video intensification microscopy, this increase was seen to be the result of expression of the receptor on promyelocytes during differentiation toward myelocytes. The receptor was never expressed on cells which remained morphologically identifiable as myeloblasts. Following binding, the peptide was rapidly internalized with a concomitant loss of surface receptors, suggesting that the peptide-receptor complex was internalized as a unit. Initial experiments indicate that 10 to 15 hours are required to replace 50% of the receptors lost from the membrane. Tunicamycin (0.1-0.5 ug/ml) blocked the increase in receptor number induced by DMF or DMSO providing evidence that the receptor may be a glycoprotein. However, treatment of the differentiated cells with sialidase, endoglycosidases or various proteases did not decrease formyl peptide binding.

452 DOES REVERSIBLE LECTIN BINDING INFLUENCE CELL GROWTH CONTROL VIA EGF RECEPTORS? Kurt Ballmer, Max M.Burger, Biozentrum der Universität Basel, 4056 Basel, Switzerland.

It has been shown earlier, that binding of the non-toxic, non-agglutinating lectin derivative succinylated Concanavalin A (SCA) to various tissue culture and primary cell lines reversibly inhibits cell growth. Furthermore it reduces the cells' ability to migrate on a solid substratum. To investigate these findings further, we used a model system where only defined growth factors but no serum is used in tissue culture. Serum starved 3T6 cells can reassume DNA synthesis and even go through mitosis if they are supplied with EGF (0.5-5 ng/ml), Insulin (100 ng/ml) and Vitamin B_{12} (400 ng/ml). Data are presented that show that SCA inhibits cell proliferation also under these conditions. Furthermore, the binding of EGF to its cell surface receptor was investigated. It turned out that only the receptor affinity, but not the total number of available sites is affected by SCA. The K_a is decreased from about $10^{12} Mol^{-1}$ to $1\text{-}3 \times 10^{11} Mol^{-1}$.

We assume that SCA interacts also with many other cell surface components which are involved in cell recognition. The observed changes in cell motility and cell growth upon SCA treatment are presumably the consequence of several molecular changes. The interaction of SCA with the EGF receptor is considered as a model system from which conclusions can be drawn to other more complicated systems.

453 CHARACTERIZATION OF THE HUMAN TRANSFERRIN RECEPTOR FROM PLACENTA AND BEWO CELLS, Caroline A. Enns, Philip E. Hass and Howard H. Sussman, Stanford University School of Medicine, Stanford, CA 94305

The physicial properties and binding characteristics of the transferrin receptor from placenta and BeWo, a choriocarcinoma cell line, were studied. Both receptors were isolated from ^{125}I-labeled membranes by solubilization with Triton X-100 and immunoprecipitation with rabbit anti-human transferrin in the presence of human transferrin. One-dimensional SDS polyacrylamide gel electrophoresis indicates a subunit molecular weight of 90,000 daltons. Although during gel chromatography on ACA-22 at 23°C in the absence of transferrin both receptors appear to be identical in size (4.9 nm), chromatography in the presence of transferrin (2 x 10^{-7}M) shows differences in mobilities; the BeWo receptor being the larger with a Stokes' radius of 6.3 nm and the placental receptor with a Stokes' radius of 5.9 nm. A combination of sucrose density centrifugation and gel chromatography give the molecular weight for the placental transferrin complex of 312,000 daltons. The effect of temperature on binding of transferrin also varies between the two receptors. Whether differences are due to differences in lipid environment, glycosylation, or separate genes, is under investigation.

454 COMPARISONS OF MICROVILLI ISOLATED FROM ASCITES SUBLINES WITH MOBILE AND IMMOBILE CELL SURFACE RECEPTORS, Robert F. Cerra, Coralie A. C. Carraway and Kermit L. Carraway, Department of Biochemistry, Oklahoma State University, Stillwater, Oklahoma 74074

Although the mobility of cell surface receptors and antigens is thought to be important to a number of cellular processes, the molecular mechanisms governing such movements are still obscure. MAT-B1 and MAT-C1 ascites sublines of the 13762 rat mammary adenocarcinoma differ markedly in cell surface antigen and Concanavalin A (Con A) receptor mobilities. MAT-C1 cells have essentially immobile receptors on a cell surface covered with numerous, highly branched microvilli. The MAT-C1 branched microvilli are stable to cytoskeletal perturbant drugs and hypotonic treatments; the unbranched MAT-B1 microvilli are not stable. We have postulated that stabilization of these microvilli is an important factor in immobilization of the surface receptors and have sought to define the stabilizing factor(s). Microvilli were obtained from the two sublines by gentle shearing in phosphate-buffered saline containing 20% fetal calf serum, purified by Percoll gradient centrifugation and characterized by enzyme analyses, cell surface labeling and electron microscopy. Polyacrylamide gel electrophoresis showed the enrichment of an Mr 58,000 polypeptide (58K) in MAT-C1 microvilli, which was not evident in MAT-B1 microvilli. Triton extraction of MAT-C1 microvilli gave residues containing predominently actin and 58K. Actin was the only major component in MAT-B1 residues. Moreover, membrane-enriched fragments of the MAT-C1 microvilli obtained by homogenization and sucrose gradient fractionation are enriched in 58K. We suggest that this component plays an important role in stabilization of the MAT-C1 microvilli and that these systems will be useful for examining molecular relationships involved in cell surface organization.

455 PHYSIOLOGICAL EFFECTS OF HIGH MOLECULAR WEIGHT FORMS OF ADRENOCORTICOTROPIN Judith C. Gasson, University of Colorado Health Sciences Center, Denver, Co. 80262

Adrenocorticotropin (ACTH) is thought to bind to specific adrenal cortical cell surface receptors and through the action of a cyclic nucleotide intermediate stimulate synthesis of glucocorticoids. High molecular weight forms of ACTH have been identified in pituitary extracts from numerous species including guinea pig; the physiological actions of high molecular weight ACTH on isolated guinea pig adrenal cortical cells were studied. Pro-ACTH/endorphin, ACTH biosynthetic intermediate and glycosylated ACTH(1-39) prepared from mouse tumor cell culture medium stimulated the same maximal production of steroid as ACTH(1-39) in the guinea pig adrenal cell bioassay. Pro-ACTH/endorphin and ACTH biosynthetic intermediate were two orders of magnitude less potent than synthetic human ACTH(1-39); glycosylated ACTH(1-39) was equipotent with ACTH(1-39). The hypothesis that the form of ACTH secreted by the pituitary determines the ratio of cortisol-related products to corticosterone-related products secreted by the adrenal cortical cells was examined. Isolated guinea pig adrenal cortical cells were incubated with the various separated forms of mouse tumor cell ACTH and the products synthesized from [^{3}H]pregnenolone were analyzed by two-dimensional thin-layer chromatography. The ratio of cortisol-related products to corticosterone-related products was the same in response to glycosylated and nonglycosylated forms of ACTH.

456 DEGLYCOSYLATION OF SURFACE ACCEPTORS PREVENTS PROGRESSION OF 3T3 CELLS FROM G_1 TO S,
Prasanta Datta, C. V. Natraj and John M. Hilfinger, The University of Michigan, Ann Arbor, MI 48109

Quiescent, but not proliferating, BALB/c 3T3 cells contain a fibroblast growth-regulatory factor (FGRF) which inhibits DNA synthesis and cell division in growing 3T3 cells by arresting them in G_1. We have found that incubation of serum-stimulated quiescent cells with purified beef kidney N-acetyl-β-D-glucosaminidase results in a transient inhibition in DNA synthesis. Regardless of the time of enzyme addition, a constant fraction of the cell population continues to enter the S phase before DNA synthesis is shut off, and the length of the "plateau period" during which no new nuclei are labeled remains constant (∿3h); upon continued incubation, cells recover and complete their progression through G_1. The rates of cells' entry into S before cessation of DNA synthesis and during recovery are similar to those seen with untreated cultures. With increasing enzyme concentrations, proportionately fewer cells enter S, however, in all cases the "plateau period" is of similar duration. When growing cells, prelabeled metabolically with ^{14}C-glucosamine, are treated with the enzyme, free labeled N-acetylglucosamine residues are released. An active component which inhibits DNA synthesis in growing 3T3 cells is also extracted from the enzyme-treated cells; further, incubation of N-acetylglucosaminidase-treated cells with uridine diphosphate-N-acetyl-D-glucosamine prior to extraction yields inactive component. We conclude that during stimulation with serum, active FGRF on the surface of quiescent 3T3 cells is replaced with an inactive form of the factor. When exposed to N-acetylglucosaminidase, the inactive component on the cell surface is converted to an active form by deglycosylation reaction and prevents the cells' entry into S.

457 BINDING AND MITOGENIC POTENTIAL OF ACTIVE-SITE MODIFIED FORMS OF α-THROMBIN TO CELLS FROM VARIOUS ANIMAL SPECIES,
Kevin C. Glenn, Darrell H. Carney and Dennis D. Cunningham, University of California, Irvine, California 92717

The interactions between regions of the active site of α-thrombin and its cellular receptor that are necessary for binding and mitogenesis were examined using four modified forms of α-thrombin. Chemical modification of the catalytic apparatus of the active site of α-thrombin by two different methods markedly reduced both fibrinogen clotting and esterase activities. Nitration or limited proteolysis of α-thrombin resulted in loss of clotting activity but retention of esterase activity. Binding and mitogenic studies were conducted on mouse embryo (ME), Chinese hamster lung (CHL), chicken embryo (CE), and human neonatal foreskin (HF) cells. α-Thrombin was mitogenic for all of these cell types; however, all four of the derivative forms of α-thrombin were inhibited in their ability to stimulate cell division. Regions of the active site outside the catalytic apparatus were critical for α-thrombin binding to all of the cell types. However, the two catalytic site-modified thrombins revealed significant differences in the α-thrombin receptor among the four cell types. These forms of α-thrombin bound in a similar fashion as α-thrombin to ME and CHL cells, but did not significantly bind to HF and CE cells. This indicates that the catalytic apparatus is required for α-thrombin binding to CE and HF but not ME and CHL cells. Since thrombins modified at the catalytic apparatus bound to ME and CHL cells as effectively as the active enzyme, this shows that thrombin-stimulated cell division requires the proteolytic activity of α-thrombin.
(Supported by NIH Grant CA-12306).

458 MOLECULAR CHARACTERISTICS AND REGULATION OF A TRANSFORMATION-SENSITIVE TRANSFERRIN RECEPTOR OF CULTURED CELLS,
J.A. Fernandez-Pol, VA Medical Center and St. Louis University, St. Louis, MO 63125

Studies were performed to identify transferrin (Tr) receptors in cultured normal rat kidney (NRK) cells. Cells were surface iodinated or metabolically labeled with radioactive glycoprotein precursors. Plasma membrane proteins were solubilized with Triton X-100. The soluble Tr receptor(s) has been purified approximately 1500-fold by a single cycle of affinity chromatography on Tr-Sepharose. On SDS gel electrophoresis the receptor proteins have iodinated components of approximately 160K and 20K daltons. Preparations of iron-fed and iron-deprived cells revealed slight differences in the MW of these proteins. Preparations subjected to two cycles of affinity chromatography revealed a single polypeptide of 20K daltons which showed specific ^{125}I-Tr binding ability. Further studies demonstrated that 20K is a degradation product of 160K glycoprotein which has been previously identified as a subunit of procollagen (J.Supramol.Struct. 1979, in press). Immunological studies showed that anti-Tr antibodies specifically precipitate a Tr-160K complex and that a specific antibody against 160K precipitates the same complex. In parallel investigations we have found a similarity between the Tr receptor of simian virus 40 or Kirsten sarcoma virus-transformed NRK cells and that of NRK cells. Studies demonstrated that Tr receptors in NRK cells are regulated by iron availability, cell density and show down regulation. These properties were found to be altered in transformed cells. The results are compatible with the hypothesis that virus-transformed cells lose some of their specific receptor sites for Tr. (Supported by VA and NIH Research Funds).

459 ALTERATIONS IN THE RESPONSIVENESS OF DIABETIC FIBROBLASTS TO INSULIN, Robert E. Fellows and Mohan K. Raizada, The University of Iowa, Iowa City, IA 52242
Fibroblastic cultures from the skin of nondiabetic and diabetic (db/db) mice have been established in order to investigate the participation of insulin receptors in the regulation of biological responses to insulin. Fibroblasts cultured from nondiabetic mice have $\sim 7.7 \times 10^4$ insulin binding sites per cell, 33% of which constitute high affinity binding sites. In contrast, cultures from diabetic mice show a 2- to 3-fold decrease in the number of insulin binding sites, without significant changes in the affinity for binding. Insulin causes a time- and dose-dependent stimulation of 2-deoxy-D-glucose (2DG) uptake in these cultures. In nondiabetic fibroblasts, 0.12 nM insulin, which inhibits ^{125}I-insulin binding $\sim$ 5%, causes a 50% increase in 2DG uptake. In contrast, a 38-fold greater concentration of insulin is required to produce 50% stimulation of 2DG uptake in diabetic fibroblasts even though the basal rate of 2DG uptake is similar in both nondiabetic and diabetic cells. Concentrations of insulin required to generate 50% stimulation of ornithine decarboxylase activity are similar to those required for 50% stimulation of 2DG uptake. Data from these and other studies indicate that fibroblasts cultured from nondiabetic and diabetic mice not only have differences in the expression of insulin receptors which are maintained over a long period of time in culture, but also express significant differences in the post receptor responses to insulin. Supported by NIH grant AM21018 to MKR.

460 DIFFERENCES IN SURFACE PROTEINS OF A TEMPERATURE-SENSITIVE RAT MYOBLAST CELL LINE, Leslie C. Engel and John D. David, University of Missouri, Columbia, Missouri 65211
A temperature-sensitive (ts) variant of the established rat myoblast line, L_6, has been isolated and partially characterized. At the permissive temperature (37°), the variant cells proceed through the normal sequence of differentiation, characterized by growth, alignment, and subsequent fusion to form myotubes. At the non-permissive temperature (40°), neither alignment nor fusion takes place. Temperature-shift experiments performed at 6 hour intervals after seeding have revealed that the temperature-sensitive event occurs at 24-36 hours, while alignment and fusion take place 5 and 8 days after initial plating, respectively. Cell surface proteins of wild-type and ts clones were labeled by lactoperoxidase-catalyzed iodination and analyzed by SDS polyacrylamide gradient gel electrophoresis. These experiments have revealed substantial differences between the two cell types. The wild-type cultures, grown at both 37° and 40°, as well as the ts mutant, grown at the permissive temperature, show similar alterations in surface proteins during myogenesis. New proteins with apparent molecular sizes of 98, 74, and 30 kilodaltons (kd) are associated with alignment. There is also an increase in the intensity of labeling of a 47 kd protein. The onset of fusion is associated with the appearance of labeled, low molecular weight proteins with apparent molecular sizes of 11 and 12 kd. Cultures of the ts mutant, grown at 40° possess neither the high nor low molecular weight proteins. Experiments utilizing mixed cultures of wild-type and ts cells have provided no evidence for the participation of a diffusable, extracellular factor in alignment and fusion, and therefore, support the hypothesis of surface protein involvement.

461 A MUTATION IN CARBOHYDRATE RECEPTORS AFFECTING CELL COMMUNICATION, Lou A. Smets, The Netherlands Cancer Institute, 1066 CX, Amsterdam.

Mouse L cells are deficient in the formation of adhesive and communicating intercellular contacts. According to many authors membrane oligosaccharides are involved in the initial steps of cell-cell adhesion and recognition. We have therefore investigated the carbohydrate moieties of membrane glycoproteins derived from L cells and somatic cell hybrids between L cells and communication-competent partners.

The results have indicated that L cells are mutant in the processing of nascent oligosaccharides. As a result, they expose at their surface high-mannosyl glycopeptides carrying many sialic acid residues. These structures may be disfunctional in cellular interactions.

The mutation in L cells can be corrected for in somatic hybrids with normal cells but only incompletely so with malignant partners. The results suggest that malignant cells are generally deficient in the synthesis of carbohydrate receptors involved in the control of the social behaviour of cells.

Ref.: L. Smets, H. van Rooy and Ch. Homburg, Exptl. Cell Res. 123, 87 (1979)

462 SPECIFIC INTERACTION OF MURINE COLONY STIMULATING FACTOR WITH MONONUCLEAR PHAGOCYTIC CELLS, L. J. Guilbert and E. R. Stanley, Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York 10461.
Mononuclear phagocytes and some macrophage cell lines have a specific binding site(s) for a subclass of the colony stimulating factors (CSFs) that specifically stimulates mononuclear phagocyte production in culture (1). Murine peritoneal exudate macrophages that had been preincubated (37°, 16 hr) in the absence of CSF were used to study the nature of the interaction of ^{125}I-L-cell CSF with its target cells. At 0° stable equilibrium binding is achieved in $\lesssim$ 20 hr ($\gtrsim$ 2 pM ^{125}I-CSF). The interaction is saturable at CSF concentrations of 1 nM. A Scatchard plot of the 0° binding data is linear ($K_d = 1.1 \times 10^{-11}$M, 5×10^4 sites per cell). A comparison of binding and competition experiments indicates that ^{125}I-L-cell CSF binds as effectively as L-cell CSF. In contrast, at 37°, ^{125}I-CSF binding does not reach a stable maximum with time and a large proportion of the bound CSF is subsequently destroyed. A two-step destruction mechanism is indicated by 37° ^{125}I dissociation experiments using cells to which ^{125}I-CSF had been bound by preincubation at 0°. The initial, rapid ($t_{½}$ 10-20 sec) commitment step can be partly blocked by the metabolic inhibitors deoxyglucose and NaN_3. This is followed by a slower ($t_{½}$ ∿ 20 min) proteolysis step which can be partly blocked by the lysosomal protease inhibitor chloroquine. The dissociated ^{125}I is either dialyzable (MW < 10K) or associated with intact (MW 70K), biologically active CSF. Partly degraded, labelled protein is not found. On the basis of this and other experiments, it appears that the binding site for CSF is a cell surface component which mediates the destruction of CSF by absorptive pinocytosis.

1. Stanley, E.R., Guilbert, L.J., Das, S.K. and Forman, L.W., this meeting.

463 BIOCHEMICAL STUDIES OF MURINE SURFACE IMMUNOGLOBULINS, Roberta R. Pollock and Matthew F. Mescher, Department of Pathology, Harvard Medical School, Boston, MA 02115
The antigen receptor of murine B lymphocytes is known to be membrane immunoglobulin (Ig) of two classes, IgM and "IgD." The physiological forms of these Igs have been studied by isolation of iodinated surface Ig on solid phase immunoabsorbents and analysis on SDS polyacrylamide gradient gels under non-reducing conditions. IgM constitutes 30% of the total iodinated surface Ig and has a molecular weight of 200,000, consistent with a μ_2L_2 structure. The remaining surface Ig is IgD, which exists in two forms, a δ_2L_2 structure (IgD_I) of 150,000 daltons and a δL structure (IgD_{II}) of 95,000 daltons. Minimizing possible proteolysis or disulfide rearrangement has no effect on the mobility or quantity of IgD_I and IgD_{II}. While the amount of spleen cell membrane IgM relative to IgD does not vary among strains, the ratio of IgD_I to IgD_{II} shows a strain variation. Using Ig-congenic mice we have shown that the IgD_I/IgD_{II} ratio is linked to the IgD allotype. IgD^a and IgD^b mice have a high ratio, while IgD^e mice have a low ratio. Thus the IgD^e allotype can be defined by a biochemical parameter in addition to its cross-reactivity with the IgD^a and IgD^b allotypes. The existence of two forms of IgD, but only one form of IgM, suggests that IgD_I and IgD_{II} may be functionally distinct, and perhaps on different subpopulations of B cells. Studies on the cellular distribution of IgD_I and IgD_{II} including data on their appearance during ontogeny, will be presented.

464 INTRA-TUMOR MATURATIONAL HETEROGENEITY OF MURINE PLASMACYTOMAS, Michael J. Daley and Noel L. Warner, University of New Mexico School of Medicine, Department of Pathology, Albuquerque, NM 87131.
A number of B cell neoplasms of man and mouse have been described which appear to represent an arrest at various stages of normal B cell differentiation. Although it has generally been accepted that these tumors are homogenous, we have found evidence to suggest that there can be significant heterogeneity within these B cell lines for certain properties. Specifically, we are interested in the maturational spectrum that can be found within many BALB/c and BALB/c.H-2 congenic derived murine myelomas. We have determined the percent of cells secreting immunoglobulin and the percent of tumor stem cells (by both in vitro and in vivo colony assays) for these myelomas. In addition, we have precisely determined by flow cytometry the quality and quantity of cell surface differentiation markers. These markers have included M.Ig, Lyb-1, Lyb-2, Ia, Pca-1 and the E2 (monoclonal antibody) defined antigen. Furthermore, we have been able in some cases to separate by biophysical methods the tumor stem cell population from the secretory population. These studies have shown that there are probably at least two distinct classes of murine myelomas. Class I contains limited maturational heterogeneity, Class II contains a spectrum of maturational heterogeneity which may include several stages of normal B cell differentiation. Current studies include an analysis of the precise maturational phenotype of the tumor stem cell to define the true spectrum of maturation and how these intra-tumor maturational events are regulated or might be manipulated by a variety of normal immunoregulatory factors.

465 STUDIES ON RECEPTORS TO CSF PRESENT ON COMMITTED HEMATOPOIETIC STEM CELLS, Dov H. Pluznik and Ruth Lenz, Life Sciences Department, Bar-Ilan University,Ramat-Gan,Israel
Committed hematopoietic progenitor cells (CFU-C) for granulocyte/macrophage differentiation proliferate in soft agar cultures to form colonies of mature granulocytes and macrophages. Colony formation is dependent on the continuous presence of a regulatory glycoprotein designated colony-stimulating factor (CSF). The in vitro assay of CSF is based on the dose response relationship which exists between CSF concentration and the number of colonies developing in bone marrow cultures. The present study was undertaken to elucidate whether CSF interacts with CFU-C via cellular receptors. In order to study this question, two experimental approaches were used: 1) to block the cellular receptor with competitive substances which are known to bind to membranal components; 2) to inactivate the CSF molecule with the receptor substance before application to CFU-C. In both approaches a decrease in the number of colonies is expected. Our initial studies showed that cholera toxin (CT), which is known for its affinity to membranal gangliosides, inhibited colony formation by CSF. Similar inhibition of the clonal growth was observed when bone marrow cells were incubated with CT before being seeded in soft agar cultures. The possibility that the observed inhibition is caused by nonspecific cytotoxic effect of the toxin was excluded by various tests. Preincubation of CSF with mixed gangliosides before addition to the soft agar cultures resulted in inactivation of the CSF activity. Pure G_{M1} gangliosides, the proposed membranal receptor for CT, neutralized the inhibitory effect of the toxin, and full recovery of CSF activity was observed. In light of these results, it is suggested that CSF exerts its effect by interaction with glycolipids present in the membrane of the committed hematopoietic progenitor cells.

466 BETA ADRENERGIC AND STEROID ACTIVATION OF ERYTHROID COLONY FORMING CELLS IN NORMAL AND MYELOPROLIFERATIVE PROCESSES, Barbara Beckman and James W. Fisher, Lab. of Hematopharmacology, Dept. of Pharmacology, Tulane Univ. Sch. Med., New Orleans, La. 70112
Cell surface receptor activation by hormones and drugs acting via cyclic AMP may play an important role in hematopoietic cell differentiation and growth. We have investigated beta-adrenergic receptor adenylate cyclase coupled activation of normal erythroid cells as well as malignant erythroid cells from mice infected with Friend virus. Evidence suggests that beta-2 adrenergic agents enhance the growth of normal bone marrow CFU-E and BFU-E derived erythroid colonies. On the other hand, beta-2 agonists inhibit malignant cell proliferation. Certain myeloproliferative disorders such as P. Vera and erythroleukemia share characteristics with murine Friend erythroleukemia in being sensitive to beta-adrenergic agents but are unresponsive to erythropoietin. Another primary area of interest of our laboratory is steroid induced erythroid progenitor cell (CFU-E and BFU-E) differentiation and the different pattern of erythroid cell response of Tfm mutant mice to steroids. The 5β-H androstanes are known to increase CFU-E in bone marrow cultures to increase CFU-E while 5α-H androstanes are not effective in increasing erythroid colony forming cells in bone marrow cultures. A marked enhancement of the erythroid colony forming cell stimulating activity of 5β-H androstanes has been demonstrated in our laboratory by changing the configuration of the 3-keto in the A ring to a 3-alpha hydroxy.

467 CHARACTERIZATION AND AFFINITY LABELING OF THE CHEMOTACTIC RECEPTOR ON DIFFERENTIATED HUMAN PROMYELOCYTIC LEUKEMIA CELLS (HL-60), James Niedel, Itzhak Kahane, and Pedro Cuatrecasas, The Wellcome Research Laboratories, Research Triangle Park, NC 27709.
Human promyelocytic leukemia cells differentiate in culture and develop formyl peptide chemotactic receptors after treatment with polar compounds (DMF, DMSO). The uptake of the potent chemotactic peptide N-formyl-Nle-Leu-Phe-Nle-(^{125}I)Tyr-Lys by these cells was rapid ($T_{1/2}$=10 min.), saturable (EC_{50} = 0.75 nM), enhanced by divalent ions and poorly dissociable. There were approximately 100,000 receptors/cell. Cells from the differentiated cultures responded chemotactically to the formyl peptides, whereas cells from the undifferentiated cultures did not. The relative potencies of five synthetic peptides to inhibit binding correlated closely with their relative potencies to induce chemotaxis, demonstrating that the measured binding was to the biologically relevant receptor. These parameters are similar to those determined for the formyl peptide receptor on human peripheral neutrophils. A protein in the differentiated cells with an apparent MW=100,000 was covalently labeled with a bromoacetylated derivative of the formyl peptide. This labeling was blocked by the underivatized peptide (250 nM) and N-formyl-Met-Leu-Phe (2 uM), but not by the non-formylated analogs which lack biological activity and do not bind to the receptor. This protein was not labeled in undifferentiated cells which lack the receptor. We believe that this protein is the formyl peptide chemotactic receptor.

The Cell Substratum

468 ALTERED GROWTH REQUIREMENTS AND DIFFERENTIATED PHENOTYPES OF CELL LINES DERIVED FROM HUMAN SQUAMOUS CELL CARCINOMAS. James G. Rheinwald and Michael A. Beckett, Laboratory of Tumor Biology, Sidney Farber Cancer Institute, Boston, MA 02115.

Human squamous cell carcinoma (SCC)--the malignant variant of stratified squamous epithelial tissues--which has traditionally been refractory to growth in culture can routinely be grown from biopsies by cocultivation with a fibroblast feeder layer. Non-stratifying epithelioid colonies of abnormal appearance arise in primary carcinoma cultures and result in established cell lines, while any normal keratinocytes in the biopsy form stratifying colonies and senesce after several passages. Cell lines have now been derived from five SCC's of the epidermis and tongue. All produce well-differentiated SCC's when inoculated subcutaneously into nude mice. The SCC lines contain 80Å keratin filaments and form intercellular desmosomal attachments, but at subnormal levels. SCC cells in monolayer culture do not synthesize the cornified envelope structure which is the ultimate marker of terminal differentiation in normal keratinocytes, but envelope formation can be triggered in a small proportion of SCC cells by suspending them in methocel-containing medium, suggesting a regulatory defect in this function.

EGF slightly alters the colony morphology of some of the SCC lines, but does not induce a higher growth rate or substantial increase in colony forming ability. The SCC lines vary in their requirement for the soluble and substratum-associated factors produced by the feeder fibroblasts; some of the SCC lines are nearly independent of them while others are as stringently dependent as normal keratinocytes. The relation between neoplastic potential and the degree of expression of tissue-specific differentiated function and response to growth factors is being examined using these lines.

469 FIBROBLAST GROWTH FACTOR MAINTAINS THE PHENOTYPIC EXPRESSION OF COLLAGEN SYNTHESIS IN CULTURED BOVINE VACULAR ENDOTHELIAL CELLS. S.C.G. Tseng, N. Savion, R. Stern, and D. Gospodarowicz. Dept. of Pathology and Cancer Research Institute, Univ. of Calif., Medical Center, San Francisco, Ca. 94143.

The different types of collagen produced by subconfluent cultures of vascular endothelial cells derived from adult bovine aortic arch and grown in the presence or absence of fibroblast growth factor (FGF) have been compared. Cultures grown and maintained in the presence of FGF (conditions uder which cells expressed their normal phenotype) synthesized primarily collagen types III and V at a ratio of 3 to 1. Collagen type V was composed of A, B, and possibly C chains. Cultures grown and maintained in the absence of FGF, (conditions under which the cells no longer exhibited their normal phenotypes), synthesized mostly collagen types III and I. The ratio of collagen type I to III and to V was 2 to 5 to 1. Basement membrane collagen (type V) was exclusively composed of A chain.

FGF could therefore modulate the synthesis of collagens which are part of the extracellular matrix (ECM) produced by vascular endothelial cells *in vitro* and could be indirectly involved in the control of the phenotypic expression of vascular endothelial cells *in vitro*.

470 CHARACTERIZATION OF BASEMENT MEMBRANE PROCOLLAGEN MADE BY HUMAN ENDOTHELIAL CELLS, Liselotte I. Fessler and John H. Fessler, Molecular Biology Institute, University of California, Los Angeles, CA 90024.

Human endothelial cells transformed with DNA fragments of SV40 synthesize type IV basement membrane collagen in culture. This procollagen is a disulfide linked heterotrimer containing two distinct proα chains, which are separate gene products, as shown by CNBr peptide mapping and by peptide mapping of the proα chains, labeled with radioactive cysteine, methionine, leucine and proline after cleavage with clostridiopeptidase or Staph aureus V8 protease. The proα chains have at least two propeptides resistant to bacterial collagenase. One peptide is tightly folded and held in its conformation by intramolecular disulfide bridges and upon reduction and denaturation it unfolds in a manner similar to that described for the type I procollagen amino propeptide (col 1). This peptide is readily labeled with leucine, tyrosine and cysteine. Another propeptide of Mol. Wt. approximately 32K is involved in the intermolecular disulfide linkage between the three proα chains.

After a 1 to 1 1/2 hour lag period the procollagen is secreted into the culture medium, but no processing of the molecule is observed. A small amount is deposited as an extracellular matrix. Cells grown in the presence of colchicine retain procollagen which is indistinguishable from that secreted into the culture medium or that deposited as an extracellular matrix. Normal human endothelial cells make the same procollagen and retain it in the cell layer, in contradistinction to the SV40 DNA transformed cells which release more into the culture medium.

471 RELATIONSHIP BETWEEN THE CYTOPLASMIC CYTOSKELETAL ORGANIZATION AND THE EXTRACELLULAR MATRIX IN HUMAN CELL HYBRIDS, Channing J. Der and Eric J. Stanbridge, Department of Microbiology, University of California, Irvine, College of Medicine, Irvine, CA 92717
The association between cell surface fibronectin (FN) and cytoplasmic microfilaments was examined in a series of hybrids formed between HeLa and three different human fibroblast strains. These hybrids are suppressed in their tumorigenic potential when assayed in nude mice. Rare tumorigenic segregant subpopulations have appeared from several HeLa/fibroblast (H/F) hybrids which are now capable of tumor formation in nude mice. Associated with this reappearance of tumorigenicity was an alteration in both cellular and colonial morphology and a striking reorganization in surface FN. The nontumorigenic H/F hybrids displayed a fibroblastic morphology with a surface FN pattern composed of incomplete, branched fibrils. The tumorigenic segregants displayed the rounded morphology of their HeLa parent with a FN organization composed of very short, unbranched fibrils localized exclusively at cell-cell junctions. This parallel shift in morphology and FN organization provided a good model system in which to analyze a possible transmembrane association between cytoplasmic actin cables and surface FN. Additionally, the relationship between microfilament organization, morphology and tumorigenicity was evaluated. Double-stain immunofluorescence was utilized to simultaneously visualize actin cables and FN. Contrary to previous observations there appeared to be no close association between these two systems. The presence of well organized microfilaments in tumorigenic segregants suggests that neither the alteration in surface FN nor the reexpression of tumorigenicity in H/F hybrids was due to a disruption of the cytoskeletal microfilament organization.

472 FIBRILLOGENESIS OF FIBRONECTIN MATRICES IN CULTURE AND IN VIVO. Margaret E. Perkins, Ian Summerhayes, Philip Hsieh, Candy Lee and Lan Bo Chen, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Mass. 02115.
Since the presence of fibronectin matrices is now well established, it is important to explore the mechanism of fibrillogenesis of fibronectin both in vitro and in vivo. For in vitro study, we used fibronectin-negative transformed cells where the fibrillogenesis of fibronectin can be initiated by the addition of fibronectin. Highly purified fibronectin dimer without proteoglycan complex is able to restore normal morphology in transformed cell but fibronectin fibers formed are very short and confined only to cell-cell contact area. However, when fibronectin-proteoglycan complex which is a minor species in 1M urea extract of chick embryo fibroblast is isolated and added to transformed cells, extensive fibronectin fibers and some fibronectin matrices can be generated. Treatment of fibronectin-proteoglycan complex with heparinase abolishes such activity and the resulted preparation behaves like purified fibronectin. We also developed a nude mice system to study in vivo fibrillogenesis of fibronectin. The tumor induced by DMBA-transformed epithelial cell in syngeneic host is fibronectin negative. However the tumor induced in nude mice is fibronectin-abundant. It appears that the extent of the presence of fibronectin matrices in tumor is proportional to the size of tumor as well as the participation of host stromal fibroblast. Thus it is possible to follow the onset of fibronectin fibers and matrices during tumor growth in nude mice and analyze factors which may be important for the fibrillogenesis of fibronectin in vivo by comparing tumor in syngeneic host and nude mice.

473 REQUIREMENT OF COLLAGEN-COATED SUBSTRATUM FOR THE GROWTH OF RAT TRACHEAL EPITHELIAL CELLS IN CULTURE, Reen Wu, Donna Smith and Ling-Yi Chang, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709

We Previously developed a hormone-supplemented medium containing 0.3% FBS, insulin, epidermal growth factor, and transferrin for the long term culture of rabbit tracheal epithelial cells. Rabbit epithelial cells grown in this condition retain some of the differentiated characteristics of cells lining the intact trachea. When we applied this culture condition to the culture of tracheal cells from other animals; such as rat and hamster, our initial results were not encouraging. It was later found that the major reason for the poor response of rat cells to this medium was because these cells require a collagenous surface for their growth. Other types of surfaces; such as serum-coated and polylysine-treated surfaces, were not suitable for rat cell cultures even though these treatments did enhance the frequency of of cell attachment. Rat epithelial cells grew best on rat tail collagen-coated dishes with an initial generation time of 24 hours and the culture could be maintained at least one month with no cellular deterioration. Other sources of collagen, such as from rat skin, calf skin, bovine Achilles tendon and the commercial product "vitrogen-100", were not found as effective as that from rat tail. Treated collagen dishes with highly purified collagenase (Worthington CLOSA product) destroyed the substrate-dependent growth of the cells. In bacterial (petri) dishes, cell growth and attachment were found dependent on the amount of collagen on the dish surface. These results suggest that the growth and maintenance of rat tracheal epithelial cell cultures is dependent on a rat tail collagen-coated substratum.

474 A SERUM FACTOR INFLUENCING SPREADING OF CELLS IN SERUM-FREE MEDIUM, D. Barnes, D. McClure, J. Orly, R. Wolfe and G. Sato, U. Cal., San Diego, Dept. Biol. 92093

A factor which stimulated spreading of cells in culture was isolated from human serum by a modification of the procedure of Holmes (J. Cell Biol. 32,297). The major portion of the protein in our preparations consisted of several bands having a mobility upon SDS gel electrophoresis consistent with molecular weights between 60,000 and 90,000. Bands representing protein of lower molecular weight were also present. The spreading activity was not lost upon incubation at room temperature with 0.1% mercaptoethanol in 6M urea but was inactivated by boiling. The serum spreading factor previously has been reported to promote growth or spreading of Hela (J. Cell Biol. 32,297) and MCF-7 human breast tumor cells (Nature 281,388) in serum-free medium (SFM). We found that this factor at 500 ng/ml promoted spreading of C6 rat glioma, WI38 human fibroblast and SV40-transformed Balb 3T3 mouse cells in SFM containing 1 mg/ml albumin. These cells did not spread well if plated in SFM with albumin alone. The factor also increased spreading of Balb 3T3 and N18TG-2 mouse neuroblastoma cells and mediated cytokinesis and growth of the RF-1 rat ovarian cell line in hormone-supplemented SFM at concentrations of 1 to 10 microgram/ml. Plasma fibronectin (CIg) was found to act in a manner similar to the spreading factor for some of these lines. However, the activity of our preparations did not appear to be due to CIg, since no CIg was detected upon SDS electrophoresis and no precipitin band was detected in immunodiffusion plates with antiserum to human CIg. This factor, active at low concentrations on a large variety of cell types in culture, may find general use in studies of cell adhesion and proliferation under serum-free conditions. Further purification is in progress.

475 THE GROWTH AND TRANSFER OF ANIMAL CELLS ON NYLON MESH, William E. Howe, Brandeis University, Dept. of Biochemistry, Waltham, Mass. 02154.

When Chinese hamster ovary(CHO) cells are added to a weave of nylon mesh(50mm diameter and mesh of 105um), approximately 5% of the cells attach to the mesh with a 5 fold variation between similary treated screens. The rate of growth of the CHO cells on the mesh is similar to that on tissue culture plates. The CHO cells transfer either from the mesh to other surfaces,plates, coverslips, or other mesh, or from other surfacer to the mesh. The rate of cell transfer from a nylon mesh can vary from 10^6 cells/month(senescence mouse cells) to 10^5 cells/12 hours with 10^7 CHO cells/screen. The mode of cell transfer from the nylon mesh is probably by cell movement at low-screen cell number and by floatation at high(10^7 cells/mesh) number.The mesh provides a 3-dimensional lattice-like-support for the cells (increases surface area 2.6 fold) which allows a layer of cells, layer of tissue, or confluent mesh to form. In a confluent mesh the CHO cells have been observed routinely to cover the weaving filaments(70um diameter) of the mesh. Therefore, the depth of the tissue can be estimated at 100um which is similar to the distance between capillaries in vivo. If the density of the cells on the mesh is calculated, it(10^8 cells/cm^3) is similar to in vivo conditions. The 3-dimensional support of the nylon mesh may allow the formation of a bed of cells similar to that between capillaries in vivo. It is postulated that different cell types and differentiated structures may be able to surivive and function in the bed of cells. Prelimary observations on mouse embryo cells support the above.

476 CONTROL OF SKELETAL MUSCLE DEVELOPMENT BY THE CELL SUBSTRATUM, Hannah Friedman Elson and Joanne S. Ingwall, University of California, San Diego, School of Medicine, La Jolla, CA 92093 and Harvard University, School of Medicine, Boston, MA 02115.

During chick embryogenesis, massive alterations occur in the migrating cell's substratum, or extracellular matrix. The possibility that some of the components of this milieu play a regulatory role in cell differentiation was explored in a cell culture system derived from embryonic chick skeletal muscle tissue. In particular, the effects of collagen and the glycosaminoglycans were studied. Collagen is required for muscle cell attachment and spreading onto plastic and glass tissue culture dishes. A major constituent of the early embryonic extracellular space, hyaluronate (HA), while having no significant effect on collagen-stimulated cell attachment and spreading, was found to inhibit myogenesis. The addition of HA inhibited the appearance of creatine kinase (CK) activity and the onset of fusion. The muscle-specific M subunit of CK was preferentially inhibited. Control experiments indicated that the inhibition was specifically caused by HA and not by other glycosaminoglycans. A general metabolic inhibition of the cultures was not observed. Muscle cells could bind to HA-coated beads at all stages of differentiation, but were inhibited only when HA was added within the first 24 hours of culture. Endogenous HA in the culture is normally degraded during the first 24 hours after plating as well; this may parallel the massive degradation of HA which occurs in the early embryo in vivo. These findings suggest a regulatory role for HA in modulating skeletal muscle differentiation, with degradation of an inhibitory component of the cell substratum a requirement for myogenesis.

477 EFFECTS OF FIBRONECTIN-RELEASING PEPTIDES ON THE EXTRACELLULAR MATRICES OF CULTURED HUMAN FIBROBLASTS. Jorma Keski-Oja and George J. Todaro, Laboratory of Viral Carcinogenesis, National Cancer Institute, Bethesda, MD 20205.

Fibronectin-releasing (10K) peptides purified from concentrated, serum-free supernatant fluids of the human fibrosarcoma cell line, 8387 (JSS 11: 217,1979) were used to study their effects on isolated cell-free matrices. Diploid human lung fibroblasts, grown to confluency, were metabolically labeled with radioactive glycine. The matrices were isolated by using sodium deoxycholate and hypotonic buffer (Hedman et al., JCB 81: 83, 1979). The major radiolabeled protein components of the matrices were fibronectin (Fn), procollagen and as yet unidentified polypeptides with mol. wts. of 180K, 140K and 66K.

The matrices that had attached to the tissue culture dishes were exposed to the 10K-peptides in medium 199. As a result of this treatment there was a massive release of both Fn and procollagen from the matrices. The polypeptides released into the media comigrated in SDS-PAGE with either the matrix-associated Fn or the procollagen chains indicating there were no major cleavages of either protein released. When the matrices were exposed to the 10K-peptides after collagenase digestion only Fn was released. In addition,the 66K protein was constantly cleaved to a 62K form that remained in the matrix. The 10K-peptides did not affect the other radiolabeled polypeptides present in the matrix. The results indicate that the fibronectin-releasing peptide behaves as a specific protease on the matrices of cultured human fibroblasts.

478 THE POSSIBLE ROLE OF EXTRACELLULAR MATRICES IN EARLY EMBRYONIC DEVELOPMENT, Angie Rizzino and Craig Crowley, Biology Dept., UCSD, La Jolla, CA 92093.

Previous studies have shown that F9 embryonal carcinoma cells can be induced by retinoic acid to differentiate in a defined medium known as EM-3 (fibronectin, insulin and transferrin in place of serum). The cells formed exhibit properties of endoderm. Once formed the differentiated cells can undergo cell division for several generations in EM-3. However, if the F9 cells are plated in EM-3 at densities lower that 5 x $10^3/cm^2$ the differentiated cells apparently form but do not survive. This suggests that the parent F9 cells, the differentiated cells or both condition their culture medium. Our data indicates that F_9 cells cultured in EM-3 release factors that bind to the culture dish and support the growth of the newly differentiated cells. If these conditioned culture dishes are used in place of the usual culture dishes, the differentiated cells formed from F_9 cells at lower densities can survive and grow, forming large clusters of differentiated cells. Like the cells formed from F_9 at higher densities, these differentiated cells have the morphology of parietal endoderm and secrete plasminogen activator. We have also obtained similar results for the growth of PYS-2 (an endoderm-like cell line with the properties of parietal endoderm) in EM-3. In consideration of the results of others, these factors might also be expected to affect the growth and/or survival of endoderm formed in mouse embryos. Work is underway to determine the nature of the factors released by F_9 in defined medium. Recently, with the assistance of Dr. G. Martin at the NIH, we have determined that F9 cells cultured in EM-3 release type IV collagen and laminin (components of basement membranes). Currently we are attempting to determine whether these molecules can substitute for the conditioned culture dishes.

479 LOCALIZATION OF RECEPTORS FOR AN ENDOGENOUS LECTIN ON CELL SURFACES AND IN EXTRACELLULAR MATRIX, Eric C. Beyer and Samuel H. Barondes, University of California at San Diego, La Jolla, California 92093

Embryonic and adult chicken tissues contain an endogenous lactose-sensitive lectin which markedly changes activity with development. The function of this lectin is presently unknown. Immunohistochemical studies have shown that the lectin is concentrated at different sites in different tissues. In developing muscle and brain, some is detectable on the cell surface of myoblasts and neurons, but most is intracellular. Lectin levels in adult muscle and brain are low, but are high in several other adult tissues. Localization differs in three adult tissues studied; the lectin is concentrated in secretory granules of intestinal goblet cells, in the extracellular matrix surrounding pancreatic exocrine lobules, and in hepatic Kupffer cells. We have localized receptors for the lectin by adding purified lectin to cultured cells and sections of fixed tissues. Lectin binding was determined by indirect immunofluorescence. Studies show that lectin binding sites are concentrated extracellularly. Cell surfaces of fibroblasts bind the lectin avidly,although these cells contain little or no endogenous lectin. Basement membranes in intestine and kidney and materials between many cell types bind the lectin. Binding was shown to be specific, since it was blocked by lactose. The implications of the finding that there are many cell surface and extracellular oligosaccharide containing materials that bind to this lectin will be considered.

480 GLANDLIKE OUTGROWTHS FROM CLONED MAMMARY CELLS ON COLLAGEN GEL, D.C.Bennett,
Imperial Cancer Research Fund,London and Salk Institute, San Diego, CA 92138
Various epithelial cells differentiate exceptionally well when cultured on the surface of a "floating collagen gel" (e.g. mammary cells; J.T.Emerman et al., Proc. Natl. Acad. Sci. USA 74, 4466 (1977)). Fragments of mouse mammary tumours can form solid outgrowths into such gels (J.Yang et al., Proc. Natl. Acad. Sci. USA 76, 3401 (1979)). Here, suspensions of "Rama 25" rat mammary epithelial cells (a tumour-derived line of apparent stem cells, which repeatedly form other cell types despite cloning; D.C.Bennett et al., Cell 15, 283 (1978)) were plated on similar gels. Over 3 weeks, multicellular structures grew from the resulting cell sheet into the gel. These structures, although often disorganised and cancer-like, included slender, repeatedly branching outgrowths, found by histology and electron microscopy to be hollow. The branches tended to develop swollen tips, as do growing mammary ducts. The outer layer of myoepithelial cells found in normal ducts was however absent from these.
Thus (a) the information to specify the growth of branching tubules can reside in a single, clonable, epithelial cell type and (b) collagen gel may be useful in studies of mammalian morphogenesis as well as cell differentiation.

481 INFLUENCE OF CELL ADHESION AND CULTURE DENSITY UPON ENDOCYTOSIS, Peter F. Davies and
Ramzi S. Cotran, Peter Bent Brigham Hospital and Harvard Medical School, Boston MA
Rate of fluid endocytosis in bovine arterial endothelial cells declined sharply when cells reached confluence and were growth-inhibited. In cultures of SV40-viral transformed 3T3 cells and SV-40-transformed human endothelial cells, endocytic rates remained unchanged with increasing culture density. Studies with quiescent 3T3 cells at different plating densities, however, suggest that there is an inverse relationship between endocytic rate and culture density independent of cell growth. Decreased penetration of tracer between and unders cells as cells per unit area increased was not responsible for these observations. When sub- and post-confluent cultures were plated onto a substratum of poly (2-hydroxyethylmethacrylate) (HEMA), cell adhesion was entirely prevented, DNA synthesis was completely inhibited and protein synthesis was partially inhibited (by 25-50%). Rates of fluid endocytosis in both 3T3 and endothelial cells 4 hours and 20 hours after plating on HEMA were elevated 3-5 fold compared with cultures adherent on a plastic surface. Such differences were reflected in transmission electron micrographs of endocytosed horseradish peroxidase. When the numbers of cells on HEMA were increased, in contrast to adherent cells, there was no decline in endocytic rates. When suspended cells were replated on plastic, endocytic rates remained elevated for up to 6 hours but declined to control levels (adherent cells) by 24 hours. These experiments suggest that for non-transformed cells, adherence to a substratum and formation of junctional complexes inhibit endocytosis.
Supported by NIH grants HL22602 and HL24612.

482 CHEMICAL INTERACTIONS OF KERATINOCYTES AND COLLAGEN, Marvin Karasek, Department of
Dermatology, Stanford University School of Medicine, Palo Alto, CA 94304
Neonatal and adult keratinocytes isolated from thin sections of split-thickness skin by trypsin-release show a preferential and strong attachment to collagen when compared to plastic, fibronectin-coated plastic, glass, or agar gels. We have investigated the reactive groups on keratinocytes and collagen required for this interaction and have determined the kinetics of attachment. At 37° both neonatal and adult keratinocytes show a rapid and irreversible attachment to collagen reaching a plateau phase at 30-60 minutes. The cells cannot be displaced from the gel by extensive washing or by conditions normally expected to break ionic bonds. Chilling to 0°C before plating completely inhibits attachment, and heating to 37°C reverses the inhibition. One cycle of freezing and thawing of cells inhibits the interaction. Removal of sialic residues from keratinocytes before plating with neuraminidase or oxidation of sugars with periodate does not inhibit attachment or growth indicating that cell carbohydrates are not required for interaction with collagen. Neither denaturation of collagen with 8 M urea or oxidation of sugar side chains on the gel with periodic acid affect attachment or growth. However, reaction of the gel with iodoacetic acid or with dinitrofluorobenzene completely inhibits growth. Blocking the guanidvl residues of collagen arginine with cyclohexanedione markedly alters all aspects of attachment, growth, and morphology producing new and completely unique growth patterns. These studies indicate that specific chemical groups on collagen affect keratinocyte-matrix interactions and that the availability of specific residues in collagen directly influences growth and maturation.

483 DEPENDENCE OF RETINOBLASTOMA TUMOR CELLS ON FIBROBLASTS FOR GROWTH, Roseline Godbout, Lily Ho and Wendy Holmes, University of Toronto, Toronto, Canada.
Retinoblastoma (RB) is an embryonal tumor that arises in children less than two years of age, prior to full retinal differentiation. It has generally been observed that RB cells do not grow in tissue sulture. At the present time, there are only two established suspension cell lines of RB. We have observed that when the cells of fresh RB tumors were placed in fibroblast monolayers, 10 out of 20 tumors could proliferate continuously. The RB cells did not grow under the following conditions: (1) without fibroblasts, (2) in Methylcellulose, (3) in Marbrook chambers with fibroblasts in the outside chamber, or (4) in fibroblast- or RB- conditioned media. Contact with the fibrobalst layer seems to be necessary for the survival and proliferation of the RB cells. After one year of growth on fibroblasts, the cells of one RB tumor have become adapted to tissue culture without a fibroblast layer. Certain fibroblast lines from RB patients appear to support RB tumor growth much better than embryonic human fibroblasts or mouse fibroblast lines.
The two main aspects of this work are (1) to understand this phenomenon of cell- contact dependence and (2) to investigate what factors are involved in the growth of RB tumor cells.

484 CELL ADHESION IN INVERTEBRATES: A POTENTIAL BASEMENT MEMBRANE COLLAGEN OF DROSOPHILA MELANOGASTER. Gregory P. Lunstrum and John H. Fessler, Molecular Biology Institute, UCLA, Los Angeles, CA 90024.
Attachment of cell sheets to a substratum should be a general feature in metazoan development. Vertebrate cells often adhere to a basal lamina (basement membrane) containing characteristic basement membrane collagens. We have found that several lines of cultured Drosophila cells incorporate radioactive amino acids into a collagen which is precipitated by cross-adsorbed antibodies made to a purified mouse basement membrane collagen (type IV). Immunofluorescence shows that this collagen is deposited around the cells. Collagen is also released into the culture media, and we have isolated an identical, or closely similar collagen from developmental stages of Drosophila. The collagen has been purified and analyzed. It consists of two types of chains which are mutually disulfide-linked into a hetero-trimer, and this can in turn associate. Each chain is larger than interstitial vertebrate procollagen chains as judged by electrophoresis and velocity sedimentation. Limited pepsin digestion leaves a disulfide-linked trimer of chains.

Synthesis of Hemoglobin, Albumin and Other Specialized Gene Products

485 THE EXPRESSION OF RAT PREPROINSULIN GENES IN SV40 RECOMBINANT MOLECULES, Peter Gruss, and George Khoury, Laboratory of Molecular Virology, National Cancer Institute, N.I.H., Bethesda, Maryland 20205.
Small DNA animal viruses have proven to be particularly valuable for studying the expression of genes in animal cells. Using SV40 as a model system, it has been demonstrated that 1) the mRNA sequence per se is not sufficient for expression of a gene and 2) deletion of sequences involved in the formation of mRNAs splices greatly reduces the formation of stable mRNA. Thus, in order to express the information of a heterologous piece of DNA inserted into the SV40 genome, sequences involved in processing of RNA of either viral or eucaryotic origin must be preserved. We have been interested in the elements controlling the expression of the two rat preproinsulin genes[1] Recombinant molecules were made in which the rat insulin genes were inserted in the late SV40 region. Experiments will be described which suggest the signals which are required for the expression of the rat gene in monkey cells.

REFERENCE:
[1]Lomedico, P., et al. (1979) Cell, Vol. 18, 545-558.

486 TRANSCRIPTION IN VITRO OF CLONED HUMAN $^{A}\gamma$-GLOBIN DNA BY HUMAN RNA POLYMERASE II,
Todd Leff[1], Frank Witney[1], Jan Hummer[1], Frederick R. Blattner[2], Stefan Surzycki[1], [1]Department of Biology, Indiana University, Bloomington, In. 47401 and [2]Laboratory of Genetics, University of Wisconsin, Madison, Wi. 53706

Details of the pattern of transcription of the β-globin genes in mammals are not clearly understood. In order to gain some insight into this process we have initiated a study of the in vitro transcription of a cloned human $^{A}\gamma$-globin gene (Smithies, O., et al. 1978 Science 202;1284) by purified human RNA polymerase II.
The positions of polymerase binding sites on the gene were mapped by electron microscopy using a protein-free spreading technique. One major binding site was observed which, in one of the two possible orientations, maps very near the sequences that give rise to the capped 5' end of the in vivo RNA. Examination of the 5' ends of RNA produced in vitro by two dimensional thin layer chromatography revealed at least one pppG starting RNA while pppA starting RNA was not detected.
In order to accurately locate the sites of the initiation of RNA synthesis, we are currently sequencing the 5' ends of the RNAs produced in vitro.

487 CONTROL OF INSULIN SYNTHESIS IN THE HAMSTER BETA-CELL LINE, HIT.
R.F. Santerre, R.A.Cook, R.D.Crisel, J.D.Sharp, R.J.Schmidt, D.C. Williams and C.P.Wilson, Lilly Research Laboratories, Indianapolis, IN. 46206
The continuous beta-cell line, HIT, was derived by SV40 transformation of Syrian hamster islet cells. Clonal lines synthesizing significant quantities of insulin have been maintained in continuous culture for more than two years (70 passages). HIT cells contain typical secretory granules and stain for insulin by indirect immunofluorescence. HIT cell insulin was extracted in acid-ethanol and quantitated by RIA, radioreceptor and bioassay. ^{3}H-leucine-labelled HIT cell insulin and proinsulin are identical to islet-derived proteins by SDS-gel electrophoresis of immunoprecipitates. Insulin release is stimulated by glucagon, IBMX and glucose. The release rate for HIT cultures was ∼2% of that for islet monolayer cultures at optimal glucose (10 mM). Somatostatin strongly inhibits HIT insulin release and synthesis. At high concentration the glucocorticoid, dexamethasone, inhibits insulin synthesis and blocks cell replication coordinately and irreversibly. Potent inducers of hemoglobin synthesis (HMBA) and interferon production (DRB) do not stimulate insulin synthesis in HIT cells.

488 THE REGULATION OF MULTIPLE ACTIN PROTEIN SYNTHESIS IN DICTYOSTELIUM discoideum.
Carol L. MacLeod and Richard A. Firtel, Univ. of Calif. San Diego, La Jolla, 92093

Actin proteins are ubiquitous, abundant, evolutionarily conserved and often differentially expressed. The D. discoideum genome contains seven actin genes. The regulation and expression of these genes has been analysed throughout the life cycle. Two-dimensional gel electrophoresis of proteins labelled in vivo and in vitro detect four separate isoforms of actin. The four isoforms are dectectable at nearly all stages of germination, growth and development. Pulse chase and re-electrophoresis experiments indicate that the actin isoforms are stable products and do not represent precursor-products of the same protein, nor are the forms likely to be a result of artifactual charge modification of a single protein. The relative amount of actin mRNA increases 300 fold during germination, remains at a high steady state in vegetative cells, and further increases from vegetative levels about 20 fold early in development, falling to low, but detectable levels late in terminal differentiation. DNA sequence analysis of plasmid inserts containing actin genes shows that at least four different actin genes are expressed. Currently, 2-dimensional tryptic peptide maps of the separated isoforms of actin are being analysed in an effort to determine whether the separated forms represent different gene products.

489 NEONATAL IMPRINTING AND HEPATIC CYTOCHROME P-450, Mark Colvin, Haiyen Chao, Robert Haas and Leland Chung, University of Colorado, Boulder, CO 80309.
Hepatic cytochrome P-450 is known to be involved in the metabolism of steroids, drugs and other xenobiotics. Substrate specificity of this enzymatic system is determined by P-450 and the presence of multiple forms of P-450 in the hepatic microsomes is well documented. Our laboratory has been interested in the purification as well as the functions of a specific sex-dependent and neonatally imprinted form of P-450. Utilizing various column chromatography steps (DEAE, hydroxyapatite and octyl-amino-Sepharose), we have partially purified this form of hepatic cytochrome P-450 to a specific content of 7.5 nmoles/mg. The major hemoprotein in the purified preparations has an apparent molecular weight of 50,000 dalton as determined by SDS gel electrophoresis. In a reconstituted system, this form of P-450 is capable of hydroxylating testosterone at the 16α-position with a turnover number of 1.4 which is about 10-fold that of the microsomal suspension. Cytochrome P-450 similarly purified from adult females or neonatal castrates failed to hydroxylate testosterone at the 16α-position whereas adult rats castrated at 4 weeks exhibited the same 16α-hydroxylase activity as that of the control males. These results suggest that neonatal androgen is responsible for the imprinting of a specific form of hepatic cytochrome P-450 which could account for the subsequent differences in substrate hydroxylation between neonatally imprinted (i.e., male or male castrated at 4 weeks of age) and non-imprinted (i.e., female or male castrated at birth) rats. Further studies on the substrate specificity and immunochemical properties of this form of hepatic cytochrome P-450 are presently in progress (supported by NIH grant GM-25027).

490 Control Of Embryonic Development: Isolation, Molecular Cloning and Quantitation of Myosin mRNAs And Of An Embryonic Inducer RNA In CHICK Heart TISSUE:

Hans-Henning Arnold, Sonia B. Jakowlew and M.A.Q. Siddiqui
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Roche Institute of Molecular Biology, Nutley N.J. 07110

Heart muscle differentiation, which is an early event in chick development, provides a model system to investigate the mechanisms controlling the transition of presumptive heart cells into well-defined myocytes. We have recently reported that a low-molecular-weight RNA from the differentiated embryonic chick heart causes the induction of heart-like differentiation in undifferentiated early embryonic cells cultivated as explants.
Attempting to investigate molecular events controlling the expression of heart muscle specific genes, we isolated and purified heart-muscle specific heavy and light chain myosin mRNAs and the inducer 7S RNA. cDNA probes complementary to the respective RNAs were prepared and amplified by molecular cloning techniques, thus facilitating the examination and monitoring of the synthesis of heart specific RNAs throughout the period of early embryonic development. Molecular interactions between 7S inducer RNA and specific gene products, like mRNA and primary transcripts of myosin genes are beeing investigated.

491 *In Vitro* Translation of the Messenger RNAs Coding for Lymphocyte Surface Antigens. Robert W. Allen, *Soldano Ferrone and James A. Hoch, Departments of Cellular Biology and *Molecular Immunology, Scripps Clinic and Research Foundation, La Jolla, CA. 92037
RNA extracted from cultured human lymphoblastoid cell lines of B-cell or T-Cell origin has been translated *in vitro* and the products reacting with xenoantisera directed against lymphocyte surface antigens have been analyzed by SDS polyacrylamide gel electrophoresis. The B-cell lines used were RPMI 6410 and Victor. The T-cell lines used were MOLT-4 and 1301.
Five polypeptides were immunoprecipitated from translations programed with RNA extracted from Victor or RPMI 6410 using two xenoantisera directed primarily against common determinants of the surface antigens coded for by the HLA-D locus of the major histocompatibility complex. The immunoreactive polypeptides have approximate molecular weights of 70,000 (p70), 68,000 (p68), 31,000 (p31), 21,000 (p21), and 19,500 (p19.5). Polypeptides p70, p68, and p21 appear to be lymphocyte specific while p31 and p19.5 are also present in immune precipitates from translations programmed with RNA extracted from either HeLa cells or the malignant melanoma cell line M-10 known to express surface DR antigens (Wilson *et al.*, J. Exp. Med. 149: 658, 1979). All of the polypeptides appear to be cell surface components *in vivo* inasmuch as reactivity to all can be absorbed from the antisera by incubation with the lymphocyte cell lines. Of the lymphocyte specific polypeptides, p68 is common to both B and T cell lines. However, p70 and p21 appear to be B-cell specific since only trace amounts of these polypeptides appear in immune precipitates of translations programmed with Molt-4 or 1301 RNA. None of the polypeptides are related to IgG since they are not competed from immune precipitates by the presence of excess unlabeled IgG.

492 SYNTHESIS AND PROCESSING OF HUMAN PRO-OPIOCORTIN, Walter L. Miller, John D. Baxter and James L. Roberts, University of California, San Francisco, California 94143
The processing of pro-opiocortin, the common precursor to corticotropin (ACTH), lipotropin (β-LPH), endorphin, and the enkephalins has been elucidated in mouse AtT20 cells. However, the genesis and processing of human ACTH is unknown, nor is it known if "ectopic ACTH", produced from certain non-pituitary tumors, arises from the same gene as pituitary ACTH. We studied the synthesis of human ACTH in tissue cultured from a pituitary adenoma and the cell-free translation products coded by mRNA from an ectopic ACTH-producing tumor. Human ACTH, βLPH and a large amino(N)-terminal fragment are cleaved from a common precursor with an M_r=35,000 on SDS polyacrylamide gels. Tryptic digests of the precursor from the two tissues yield identical peptides, suggesting that pituitary and ectopic ACTH are generated from identical precursors. Tryptic mapping also indicated the cleavage sites on the precursor peptide are the same as those previously deduced for murine and bovine ACTH. Immunoprecipitation and SDS polyacrylamide gel electrophoresis of the translated peptides suggests the primary structure of the precursor is similar in all three species. However, antibodies to the murine N-terminal fail to precipitate the human N-terminal. Two-dimensional gel electrophoresis of peptides labeled in vivo with ^{35}S-met or ^{3}H-glucosamine indicated that glycosylation of the human ACTH precursor is very different from the murine precursor. Human ACTH precursor is glycosylated only in the N-terminal fragment, not in the ACTH and βLPH sequences as in the mouse. These data suggest 1) ectopic and pituitary ACTH arise from the same gene; 2) regulation of processing in man and mouse differ; 3) the N-Terminal, which may be a hormone, may differ in mouse and man.

493 SYSTEM FOR THE STUDY OF HUMAN ALPHA GLOBIN(HAG) GENE EXPRESSION, A. Deisseroth, U. Bode, J. Fontana and D. Hendrick, Exp.Hem. Sect., NCI, NIH, Bethesda, Md., 20014.
In order to study genetic mechanisms governing differentiated gene expression in human hematopoietic cells, we have exploited the linkage of human alpha globin(HAG) genes to a selective marker, adenine phosphoribosyl transferase of human chromosome 16, to isolate hybrid mouse erythroleukemia(MEL) cells which specifically retain this chromosome while losing most or all of the other human chromosomes. Hybrid diploid MEL cells which retain this chromosome from erythroid cells produce HAG chains as well as HAG mRNA. In hybrid diploid MEL cells which have the same chromosome from non-erythroid human hematopoietic cells, no HAG chains can be detected, and only very low levels of HAG mRNA are present. Thus, we have developed a system in which the expression of a differentiated human gene(alpha globin) correlates, after transfer to a mouse cell, with the differentiated state of the human donor cell. Our work in this system suggests that the restriction of HAG gene expresison in non-erythroid cells arises from a mechanism which acts in cis to human chromosome 16 and which is able to maintain this restriction after many passages in the MEL cell. Experiments in hybrid MEL cells containing a tetraploid complement of mouse chromosomes as well as HAG genes from non-erythroid human hematopoietic donor cells have shown that the MEL cells contain a factor that is capable of reactivating full expression of HAG genes derived from non-erythroid(undifferentiated) human donor cells. These results suggest that study of the pattern of expresison of HAG genes derived from cells of different states of differentiation in a differentiated recipient cell, such as the MEL cell we used in our studies, could potentially clarify which regions of a gene are important to its regulation in differentiated and undifferentiated cells.

494 STUDIES ON THE EXPRESSION OF THE EPSILON HEAVY CHAIN GENE OF RAT IgE, Wayne Kindsvogel, Joan Moore and Charles H. Faust, Jr., University of Oregon Health Sciences Center, Portland, OR 97201
The mRNA coding for epsilon heavy chain has been highly enriched from a rat IgE-producing myeloma, IR-162, using standard physicochemical procedures. The epsilon heavy chain mRNA runs at 20S in a sucrose velocity gradient. Using denaturing gel analyses, this mRNA has an estimated molecular weight of 900,000 daltons--equivalent to about 2650 nucleotides. Only about 60% of the length of this mature rat cytoplasmic mRNA codes for protein. The mRNA is estimated to be about 40% homogeneous by kinetic complexity analysis. The epsilon heavy chain mRNA is active in an mRNA-dependent cell-free protein synthesizing system. This mRNA stimulates the synthesis of a single major protein which is serologically related to authentic heavy chain of the secreted rat IgE. It is unglycosylated and has an apparent molecular weight of 62,000 daltons, compared to 76,000 daltons for the glycosylated in vivo heavy chain of secreted IgE. The unglycosylated in vivo epsilon heavy chain obtained in the presence of tunicamycin has an apparent molecular weight of 58,000 daltons. Therefore, the in vitro synthesized epsilon heavy chain protein is about 4,000 daltons larger than the in vivo unglycosylated heavy chain. This is less than 40 extra amino acids, and is consistent with an epsilon heavy chain putative precursor. These extra amino acids may be located on the amino and/or carboxy termini. The 20S epsilon heavy chain mRNA has been used as a template for the synthesis of cDNA and double-stranded cDNA. The double-stranded cDNA is being used for cloning via recombinant DNA technology to examine epsilon heavy chain gene structure and organization.

495 CLONING OF HUMAN ACTIN GENES, Joanne Engel and Laurence H. Kedes, Howard Hughes Medical Institute at Stanford Medical Center, Stanford, CA. 94305.

Study of the genomic organization and expression of the human actin genes will illuminate the control of multigene families during development and differentiation. As a first step towards understanding the control of this gene family, we have been attempting to isolate the human actin genes by recombinant DNA techniques. Using two different actin clones derived from the D. Melanogaster genome as probes, discrete bands were identified on Southern blots made from total human DNA digested with various restriction enzymes. These probes have been further used to screen a library of human DNA cloned into bacteriophage CH4A. Eight clones were selected containing inserts of appropriate sizes that are homologous to the coding region of the two cloned Drosophila actin gene probes. These clones are currently being tested for the ability to selectively hybridize to human actin mRNA.

496 EFFECT OF HEME ON GLOBIN mRNA SYNTHESIS IN SPLEEN ERYTHROID CELLS, P. Ponka*, O. Fuchs, J. Borova and J. Neuwirt, McGill University*, Montreal, Canada and Charles' University, Prague, Czechoslovakia.

Synthesis of globin mRNA in erythroid spleen cells from anemic mice was measured after in vitro incubation under conditions in which the level of intracellular heme was manipulated. This newly synthesized globin mRNA was isolated by hybridization with globin cDNA covalently bound to cellulose. INH and penicillamine were used as specific inhibitors of heme synthesis. It has been found that a 120-min incubation of spleen erythroid cells with 5mM INH or 5mM penicillamine reduced (^{3}H) uridine incorporation into globin mRNA by 24% or 36%, respectively. The addition of heme to INH- or penicillamine-treated cells almost completely restored (^{3}H) uridine incorporation into globin mRNA. These results indicate that heme stimulates transcription or processing of globin mRNA.

497 ANALYSIS OF HEMOGLOBIN SWITCHING IN SHEEP, Arthur W. Nienhuis, Jane E. Barker, and Peter Kretschmer, NIH, Bethesda, MD 20205

Our studies are directed toward the analysis of hemoglobin switching using the sheep as a model. As determined by in vivo experiments and analysis of hemoglobin synthesis by erythroid colonies, induction of Hb C ($\alpha_2\beta^C_2$) appears to be mediated by erythropoietin acting on an erythroid stem cell at the CFU-E stage. With respect to the fetal (Hb F = $\alpha_2\gamma_2$) to adult (Hb A = $\alpha_2\beta^A_2$) or Hb B = $\alpha_2\beta^B_2$) hemoglobin switch in sheep, the stem cells which form erythroid colonies in vitro appear to be precommitted in vivo with respect to hemoglobin phenotype: BFU-E and CFU-E from the fetal sheep give rise to colonies making Hb F while CFU-E from adults give rise to colonies making adult hemoglobin. Our molecular analysis of hemoglobin switching has utilized molecular cloning to isolate globin genes. Characterized to date are the ε (early embryonic), γ (fetal) and β^A (adult) globin genes. Each contains small and large intervening sequences as determined by restriction endonuclease mapping and limited DNA sequencing. Heteroduplex mapping by electron microscopy has revealed extensive homology in the regions flanking the γ and β^A globin genes. The large intervening sequence from the ε, γ, and β^A globin genes have been subcloned into plasmids. Southern blotting analysis suggest only limited homology between these DNA fragments. Thus they should be suitable as probes to determine the presence and relative concentration of mRNA precursor molecules and facilitate further definition of the molecular mechanism of selective accumulation of various globin mRNAs during development.

Role of Proteases in Growth and Development

498 INHIBITION OF CELLULAR PROTEASE ACTIVITY AND PROLIFERATION BY CARTILAGE DERIVED FACTORS AND HEPARIN. Victor B. Hatcher, Albert Einstein College of Medicine, Montefiore Hospital & Medical Center, New York, N.Y. 10467

The molecular events in the inhibition of cell proliferation by cartilage derived factors and heparin are not clearly understood. Cartilage derived factors containing trypsin inhibitory activity but not papain inhibitory activity inhibits protease activity associated with the cell surface and cell proliferation of rat fibroblasts, smooth muscle cells and transformed mouse epidermal cells. Commercial heparin also inhibits protease activity associated with the cell surface and cell proliferation of rat smooth muscle cells. The inhibition of cell surface protease activity and cell proliferation by heparin was abolished when: (1) heparin was treated with protamine sulfate; (2) acid treated serum was used; (3) acid treated serum and human antithrombin III were used. Protease activity associated with the cell surface is related to cell proliferation [1,2]. One of the early events which occur when cartilage factors and heparin inhibit cell proliferation in normal and transformed cells is the inhibition of cell surface protease(s). References:
(1) Hatcher, V.B., Wertheim, M.S., Choo, R.R., Tsien, G. and Burk, P.G. Biochem. Biophys Acta. 451:499-510, 1976 (2) Hatcher, V.B., Oberman, M., Wertheim, M.S., Tsien, G., Rhee, C. and Burk, P.G. Biochem. Biophy. Res. Comm. 76:602-608, 1977
(Supported in part by grants AG01732 and HL16387 from NIH, New York Heart Association and Cystic Fibrosis Foundation).

499 CHARACTERIZATION OF A PLASMA MEMBRANE ASSOCIATED PROTEASE (PLASMINOGEN ACTIVATOR) ON THYMOCYTES. R. Jerrold Fulton and David A. Hart, UTHSCD, Dallas, Texas 75235.
Proteolytic events have been implicated as mechanistic steps in a variety of cellular regulation functions, including the control of cellular proliferation in lymphocytes and other cell types. Little information is available concerning the endogenous proteases which could be involved in lymphocyte proliferation except that they appear to be either secreted or cell surface enzymes. We have previously characterized the cell surface proteases on viable thymocytes, and in this report, we have extended the biochemical characterization of the proteases which are associated with the plasma membrane (PM) fraction of thymocytes. A protease has been identified which is specifically localized to the PM fraction. This protease appears to be both functionally and biochemically distinct from the intracellular proteases which are present in thymocytes since it has unique molecular properties and represents the major thymocyte protease which can function as a plasminogen activator (PA). The PM associated PA appears to be a glycosylated serine protease of 105,000 MW. The enzyme can be detected botn at the surface of viable thymocytes and in the isolated PM fraction. The sensitivity of the PM associated PA to a variety of protease inhibitors was determined in order to examine the activity of this protease under conditions which inhibit lymphocyte proliferation. While some correlations were obtained, the currently available evidence does not support the possibility that the PM associated PA is directly involved in the regulation of lymphocyte stimulation.

500 EVIDENCE THAT A PROTEOLYTIC EVENT(S) IS INVOLVED IN LYMPHOCYTE ACTIVATION. Joan Stein-Streilein and David A. Hart, UTHSCD, Dallas, Texas 75235.
Proteases have been shown to activate murine, human and hamster lymphocytes, augment B-cell mitogenesis (hamster), as well as subsititue for T-cell help (mouse). Conversely, reports have demonstrated that soybean trypsin inhibitor (SBTI) or α-tosyl-L-lysyl-chloromethane (TLCK) can suppress lymphocyte mitogenesis, (hamster, mouse, guinea pig) and that alpha-1 anti-trypsin inhibitor (α1-AT) can limit the development of antibody forming cells (AFC) to sheep erythrocytes (SRBC) (mouse). It was the intention of these studies to further evaluate the hypothesis that a proteolytic event might participate in an antigen dependent proliferation and differentiation response. Therefore, a variety of commercially available proteases and protease inhibitors were examined for their ability to modulate a normal in-vitro AFC response of hamster lymph node or spleen cells to SRBC using a modified Mishell-Dutton assay. Certain (trypsin and papain) but not all proteases tested (α chymotrypsin, thrombin, submaxillary proteases) were able to effect an enhancement of the AFC response to SRBC. Optimal enhancement (2-10 fold) of the response was only possible if the enzyme was added at the initiation of the culture. Modulation of the AFC response by competitive protease inhibitors provided more direct evidence that a proteolytic event might be a normal component of the proliferation mechanism. Protease inhibitors added at the initiation of the cultures led to a greater than 50% reduction of AFC. Addition of the inhibitor 24 to 48 h later did not modulate the AFC response or demonstrate any toxicity of the chemicals on the cells. These data support our hypothesis that a natural second signal needed for specific activation of lymphocytes involves a proteolytic event.

Lymphocyte Triggering

501 BLAST-TRANSFORMATION OF HUMAN B-LYMPHOCYTES BY EPSTEIN-BARR VIRUS, Bill Sugden and Willie Mark, McArdle Laboratory, University of Wisconsin, Madison, WI 53706
Epstein-Barr Virus (EBV) induces and maintains blast-transformation in human B-lymphocytes. We have found that a single particle of the virus is sufficient to effect this change and that its DNA is amplified sometime after infection in the blast-cells. Other workers have found that the bulk of viral DNA in EBV-induced blast-cells consists of extra-chromosomal, circular molecules (Lindahl et al. (1976) J. Mol. Biol., 102: 511-530). We have measured the apparent target size with γ-rays for blast-transformation to be equal to all of the DNA of the virus. In addition we have measured the apparent target size using γ-rays for the primary induction of cell DNA synthesis in human B-lymphocytes by EBV. Again all of the viral DNA appears to be required for this early event in the conversion of a lymphocyte to a lymphoblast. The major unrepaired lesion induced by γ-rays in DNA is a double-strand scission. We interpret our findings to indicate that: 1) transcription across the joint region formed by intracellular circularization of the linear virion DNA is required for blast-transformation; or 2) viral DNA synthesis is required for blast-transformation; or 3) all viral information must be expressed for blast-transformation. We are now attempting to test these interpretations to ascertain if any or all are valid.

502 THE INTERACTION OF LIPOPOLYSACCHARIDE AND MOUSE SPLEEN CELLS, Allen Rosenspire and Diane Jacobs, Department of Microbiology, S.U.N.Y. at Buffalo, Buffalo, NY 14214
Lipopolysaccharide (LPS) is a substance which has a variety of known biological effector functions. Among these is its ability to be mitogenic to most, but not all, strains of mouse B cells. The detailed molecular mode of action is in all cases presently unknown, although presumably LPS initially interacts with the membrane or a membrane component of sensitive B cells.

We report here on our efforts to define the mode of action of LPS with mouse spleen cells. In particular by the use of a radiolabeled LPS of very high specific activity, we have been able to show that LPS interacts with these cells in at least two different ways. We show that it interacts with cell membranes in a manner essentially analogous to polypeptide hormones, in that there seem to be specific high affinity receptors present on the membrane that account for a portion of the total LPS-cell binding. We estimate that the LPS-LPS receptor interaction is characterized by a binding constant of about 5×10^7 M/L, with an average of 4×10^3 binding sites per cell, taken over a typical whole spleen cell population.

The second (and dominant) mode of binding is either nonspecific, or else the result of binding to a receptor of much lower affinity ($<10^5$ M/L).

We find that specific LPS receptors probably appear on cells of nonresponding mouse strains, as we find no essential difference in the specific binding of LPS between responder and nonresponder strains. This implies that the nonresponding lesion, which has been shown to be the result of a single mutation, is not a reflection of an altered reception process, but concerns a step in B-cell activation after LPS binding.

503 ROLES OF CELL SURFACE IgM and IgD IN THE POLYCLONAL ANTIBODY RESPONSE TO ANTI-IMMUNOGLOBULIN AND A T CELL-DEPENDENT SOLUBLE FACTOR, Dr. David C. Parker, University of Massachusetts Medical School, Worcester, MA 01605
Cultures of isolated mouse splenic B lymphocytes activated by the divalent $F(ab')_2$ fragment of purified rabbit anti-mouse Fab or class specific anti-mouse IgM antibodies can be driven on to high rate immunoglobulin secretion by the addition of a cell-free, 24-hour culture supernatant of concanavalin A-activated spleen cells (Con A SN). The polyclonal antibody response to anti-Ig plus Con A SN is comparable in magnitude to the LPS response as measured by a reverse plaque assay. Addition of Con A SN can be delayed for 24 hours after anti-Ig without changing the kinetics of the response. Addition at 48 hours delays the response by 24 hours. The response to $F(ab')_2$ anti-Fab plus Con A SN appears to be sensitive to Fc-dependent inhibition, since intact anti-Fab antibodies inhibit strongly at relatively low concentration. The monovalent Fab' fragment fails to activate, indicating that cross-linkage of surface immunoglobulin is required. Although the production of active Con A SN is T-dependent, the response to anti-Ig plus Con A SN is T-independent. Similar experiments with class-specific rabbit anti-mouse IgD will determine whether ligand interactions with surface IgD have different effects on cell proliferation and differentiation to immunoglobulin secretion in this polyclonal model of the antibody response.

504 XENOSERUM-INDUCED PROMOTION OF POLYCLONAL T AND B CELL RESPONSES, P. Golstein, R.B. Taylor, F. Denizot and B. Rubin, Centre d'Immunologie de Marseille-Luminy, Case 906, 13288 Marseille Cedex 2, France.

When cells from mice primed in vivo with xenogeneic serum (xenoserum, for instance fetal calf serum) are boosted in vitro by incubation for 5 days in the presence of the same xenoserum, both polyclonal T and B cell responses develop. An analysis of this phenomenon showed that (1) xenoserum triggers the appearance of xenoserum-specific "promoter" (probably T) cells, (2) these promoter cells, irradiated, are able to induce the differentiation of fresh normal T and B spleen cells, via soluble factors released from the promoter cells, and (3) this differentiation leads in particular to the appearance of polyclonal cytolytic T cells (with, interestingly, anti-self components) and plaque-forming descendants of B cells. It is not yet known whether the same promoter cells and factors are able to act on both T and B cell compartments. Further details will be presented on this experimental system, which involves soluble factors probably made by lymphocytes and inducing the differentiation of other lymphocytes from precursor to effector cells in the absence of the corresponding antigens.

505 MINIMAL CONDITIONS FOR THE ACTIVATION OF SINGLE CYTOTOXIC PRECURSOR, Hung-Sia Teh and Soo-Jeet Teh, Department of Microbiology, University of British Columbia, Vancouver, Canada V6T 1W5

The cellular requirements for the activation of cytotoxic T lymphocytes (CL) to alloantigens are complex. In addition to the cytotoxic precursor (CLP), metabolically active allogeneic stimulator cells, adherent A cells, and helper T cells are also required. However, the requirement for adherent A cells, metabolically active stimulator cells, or helper T cells can be replaced by costimulator, a lymphokine obtained by stimulation of murine spleen cells with Con A. This suggests that the minimal conditions for the activation of CLP are alloantigen, and a non-antigen specific inductive signal. In agreement with this hypothesis, we found that culture conditions limiting for CLP are achieved when we supplemented our limiting dilution cultures with costimulator. In an attempt to show that the cytotoxic precursor is a target cell of costimulator we proceeded to determine if costimulator can activate cultures containing an average of one lymph node (LN) cell to from CL. This experiment is rendered feasible by substituting Con A, a polyclonal activator of CLP, for alloantigen. Under these conditions, out of 240 cultures containing on the average one $H\text{-}2^b$ LN cell, Con A, and costimulator, the number of cultures giving rise to detectable cytotoxic clones for 3 different experiments were 7, 11, and 6, respectively. In all 3 experiments, the number of responders for cultures containing 1, 3, or 10 $H\text{-}2^b$ LN cells observed were within those predicted by Poisson statistics. A log-log plot of cell dose vs lytic activity/culture was linear and gave a slope of one. These studies therefore suggest that one of the target cells of costimulator is the CLP.

Supported by the National Cancer Institute and the Medical Research Council of Canada.

506 GENERATION OF A LYMPHOCYTE GROWTH FACTOR BY CELLS ACTIVATED BY MITOGEN OXIDIZING AGENTS, Abraham Novogrodsky, Manikkam Suthanthiran, Albert L. Rubin, Kurt H. Stenzel. Cornell University Medical College, New York, N.Y. 10021. The oxidizing mitogens induce lymphocyte activation by generation of aldehyde moieties on cell-surface glycoproteins. They thus provide a useful tool for the study of mediators of lymphocyte activation, since brief incubation of cells with these agents is sufficient to generate the mitogenic signal, and the oxidizing agent can then easily be removed. Supernatants of lymphocytes treated with neuraminidase-galactose oxidase (NAGO) were found to induce blastogenesis in non-proliferating cells harvested 7-10 days after treatment with Con A or NAGO (mitogen-induced memory cells) and in cells 14 days following initiation of a primary MLC (MLC-memory cells), but not in freshly isolated peripheral blood lymphocytes (PBL). Virtually all the growth factor was produced by NAGO treated cells during the first 24 hrs of incubation and no increase in factor activity was detected upon further cell culture. Serum is not required for growth factor production. The growth factor has little effect on proliferation of lymphocytes stimulated with different concentrations of mitogens or on proliferation of primary MLC's. The growth factor induced the generation of specific cytotoxic lymphocytes from MLC-memory cells to approximately the same extent as did allogeneic cells (stimulating cells in the 1°MLC). Generation of cytotoxic lymphocytes in the 1°MLC was not affected by the growth factor. Growth factor activity could be absorbed with NAGO-induced blasts, and NAGO-induced and MLC memory cells, but not with PBL. Molecular properties of the NAGO-generated growth factor and its relation to other lymphocyte-generated growth factors are currently under investigation.

507 A NEW CLASS OF INHIBITORS OF LYMPHOCYTE MITOGENESIS: AGENTS THAT INDUCE ERYTHROID DIFFERENTIATION IN FRIEND LEUKEMIA CELLS, Kurt H. Stenzel, Albert L. Rubin and Abraham Novogrodsky, Cornell University Medical College, New York, N.Y. 10021.
Polar organic compounds, such as dimethylsulfoxide, N,N-dimethylformamide, N,N-dimethylacetamide and n-butyric acid, known to induce erythroid differentiation in Friend Leukemia cells, markedly inhibit human lymphocyte activation induced by the tumor promoting agent, phorbol myristate acetate (PMA), without affecting cell viability. Inhibition was assessed by tritiated thymidine, leucine and uridine incorporation. Morphological transformation was also inhibited by the organic agents. Inhibition is achieved at concentrations of the organic compounds reported to be optimal for induction of erythroid differentiation. Compounds that are structurally related to butyric acid, but that do not induce erythroid differentiation, do not inhibit, lymphocyte mitogenesis. Mixed lymphocyte cultures were as highly sensitive to inhibition by these agents, as were PMA-induced responses. Lymphocyte responses to other mitogens, such as phytohemagglutinin (PHA), concanavalin A and neuraminidase-galactose oxidase treatment, are also suppressed by the polar organic compounds, although higher concentrations are required. Cultures stimulated with PMA and PHA together resemble those stimulated with PHA alone, in terms of their resistance to inhibition by the polar organic compounds. Inhibitory activity was not detected in culture medium or sonicates of cells treated with PMA and the organic compounds. PMA stimulation of lymphocyte preparations depleted of adherent cells is also inhibited by the organic agents. These compounds are much less inhibitory when added 24 hours after initiation of the cultures. Preliminary data suggest that inhibition is mediated via interference with the mitogenic signal at the membrane level.

508 THE PROGENY OF EARLY RESPONDING B LYMPHOCYTES HAVE A DIFFERENT PROLIFERATIVE BEHAVIOR THAN THE PROGENY OF T LYMPHOCYTES. Allen J. Norin. Montefiore Hospital Albert Einstein College of Medicine, New York, N.Y. 10467.
In most schemes of B lymphocyte differentiation proliferation is an essential feature of the maturation process. B cells may display a number of possible modes of growth and differentiation after activation by a polyclonal mitogen such as Lipopolysaccharide (LPS). For example, multiple divisions of individual parental B lymphocytes may give rise to expanded clones of antibody secreting cells. Alternatively, some B cells may undergo a limited number of divisions and then terminal differentiation to antibody secretion. In order to study mitogen independent cellular metabolism in the progeny of cells that have previously been activated, the stimulant must be removed from the cell surface since residual bound mitogen might induce a response. I found that cells required at least 4 washes with medium to remove mitogenic quantities of LPS from the cell surface. Mitogen stimulated lymphocyte cultures are asynchronous with respect to when cells enter an initial S phase thus by conventional methods it is difficult to follow daughter cells through successive divisions. Replicated DNA molecules can be recognized when a single strand of DNA in an initial duplication is radiolabelled and the opposite strand tagged with the heavy precursor, 5 BrdU, in a subsequent S phase. Most B lymphocytes that undergo an initial cell division during the first 48 hours of culture undergo at most two cell divisions the second of which is mitogen independent. In contrast, the progeny of many of the T lymphocytes that respond to concanavalin A during the first two days continue to proliferate but they require further signalling at the cell surface.
Supported by USPHS grant CA22088 and HL17417.

509 A Genetic Defect in the Macrophage-B Lymphocyte Interaction Required for Anti IgM Stimulated Proliferation.

Henry H. Wortis, Patricia Mongini, Tufts University School of Medicine, Boston, Massachusetts 02111

Splenic B cells can be stimulated to proliferate by goat anti mouse μ. The responding cells are in the Lyb_3 bearing subpopulation. Treatment of spleen cells with silica or removal of adherent cells abrogates the response. Lymph nodes are deficient in accessory cells. Addition of irradiated plastic adherent cells or 2 mercaptoethanol (2ME) to lymph nodes or depleted spleen cells permits proliferation. Adherent cells from the spleen and peritoneum differ in their ability to support responses. Mice from several strains including C3HeB/FeJ fail to respond. This failure can be overcome with adherent cell supplementation or 2ME. Thus the defect appears to be due to a failure in an interaction between Lyb_3 positive B cells and macrophages. The defect is due to recessive alleles at an autosomal locus (not LPS).

The antibody response of Lyb_3 positive B cells depends on macrophages as does proliferation in response to anti μ. Furthermore Lyb_3 positive cells, unlike other B cells, bind to macrophages. Thus it appears that Lyb_3 positive B cells enjoy a special relationship with macrophages.

510 EVIDENCE FOR THE INFLUENCE OF c-AMP ON MEMBRANE-CYTOSKELETON INTERACTIONS IN LYMPHOCYTES. Coralie A. Carothers Carraway and Kermit L. Carraway, Department of Biochemistry, Oklahoma State University, Stillwater, Oklahoma 74074.
Previous studies on ascites tumor cells and fibroblasts have shown that the ecto enzyme 5'-nucleotidase is responsive to changes in membrane-cytoskeleton interactions. Extraction of actin and associated cytoskeletal proteins from ascites cell surface membranes causes the inhibition of 5'-nucleotidase by Concanavalin A (Con A) to become cooperative. Treatment of ascites cells or fibroblasts with cytoskeletal perturbant drugs (colchicine, cytochalasins, dibucaine, ionophore and calcium) also induces cooperativity. This effect can be prevented by theophylline or dibutyrylcyclic AMP, drugs which raise cellular cyclic AMP levels. In contrast to fibroblasts and ascites cells splenic lymphocytes exhibit cooperativity in the inhibition of 5'-nucleotidase by Con A. Treatment of the lymphocytes with 1 mM theophylline or dibutyrylcyclic AMP prevents the cooperative response. This concentration of these drugs is required to raise the cellular cyclic AMP levels and will also inhibit blastogenesis by Con A. Under the same conditions 0.1 mM drugs alter neither cooperativity nor blastogenesis. We propose that the changes in cell surface nucleotidase reflect membrane-cytoskeleton alterations which are sensitive to cyclic AMP and suggest that these alterations may play a role in the complex mechanisms governing lymphocyte proliferation.

511 THE ABILITY OF INSULIN TO INFLUENCE MURINE T LYMPHOCYTES RESPONDING TO MITOGENIC STIMULATION IN VITRO, E. Charles Snow, Thomas L. Feldbush, and John A. Oaks, University of Iowa, Iowa City, Iowa 52242
The insulin receptor is not phenotypically expressed upon the surface of resting murine B and T lymphocytes. During both the Concanavalin A (Con A) and phytohemagglutinin (PHA)-mediated blast transformation with murine lymphocytes, there is a demonstrable appearance of cell surface receptors for insulin. This specific insulin receptor is not expressed on the lymphocyte's surface until at least 12 hours following lectin-activation of the cell, but precedes the DNA synthesis associated with cell division. The results to be presented indicate that the presence of insulin, in a serum substitute system, enables mouse splenic lymphocytes to respond to Con A in a fashion similar to control cells stimulated by the lectin in the presence of fetal calf serum. Furthermore, once the splenic lymphocytes have been activated by a short-term exposure to Con A, insulin can replace the Con A in stimulating both mitogenicity and the nonspecific generation of cytotoxic effector cells.

512 TUBULORETICULAR INCLUSIONS ARE FORMED WHEN TRIGGERED BY A BUdR INDUCED HUMAN LYMPHOID CELL FACTOR, Steven A. Rich, N.Y.S. Dept. of Health, Div. of Labs. and Research, Albany, N.Y. 12201.

Tubuloreticular inclusions (TRI) are a pathological curiosity formed within the endoplasmic reticulum of endothelial cells and peripheral blood lymphocytes of patients with autoimmune, immunodeficiency and certain neoplastic disorders. Ultramorphologically they appear virus-like. Raji, a human B lymphoblast cell line, forms these structures (as early as 36 hrs) upon 5-bromo-2'-deoxyuridine (BUdR) substitution for thymidine in their DNA. We have now found that non-BUdR substituted Raji cells form TRI (as early as 21 hrs) when grown in media conditioned by the growth of Raji cells which had had their DNA unifilarly substituted with BUdR. Conditioned media treated with charcoal, diluted 1:1, ultrafiltered (0.2 u Nalgene), ultracentrifuged (100,000 X g, 60 min), or heated at 56°C for 30 min retained the inducing activity. Conditioned media diluted 1:9 no longer caused the formation of TRI. Together these results suggest that the conditioned media activity is not due to residual cells or bacteria (ultrafiltration), virus particles (ultracentrifugation), a nucleotide (charcoal) or a nucleo-protein complex (56°C, 30 min). The results are consistent with a BUdR-induced human lymphoid cell factor of a peptide nature, at an above critical concentration (1:1 vs 1:9 dilution), that can trigger a human B lymphoblast to undergo the formation of these inclusions. Interest in the effect of this factor on lymphoblasts and the formation of TRI is derived from the apparent immunological role of lymphoblasts in the above mentioned disease conditions.

513 ROLES OF TWO HELPER T CELLS IN THE B CELL RESPONSE TO PROTEIN-BOUND ANTIGENS,
Daniel M. Keller, James Swierkosz, John Kappler, and Philippa Marrack, University of Rochester, Rochester, New York 14642
Evidence is presented for a two-signal model of T cell helper function in the B cell response to the protein antigen TNP-keyhole limpet hemocyanin (TNP-KLH). Our results suggest that antigen-specific and nonspecific helper signals may be involved in different stages of the B cell response. Signal 1 appears to be antigen-specific and is required for initiation of B cell proliferation in response to antigen. It can be provided in culture by KLH-primed T cells (apparently, Ia^-), but thus far we have been unable to produce a cell-free product that can deliver this signal. Results suggest that macrophages preincubated with KLH and KLH-primed T cells can provide this activity to cultures. This signal, therefore, may be presented to B cells indirectly via T cell-macrophage interaction.
Signal 2 is present in a 24 hour supernatant of Con A stimulated spleen cells, which suggests that it is non-antigen-specific. Both signals 1 and 2 are required for a response. Using appropriate recombinant strains, a major determinant on the T cell providing signal 2 was shown to be I-A.
Whereas signal 1 must be present from the beginning of the response, signal 2 may be added to cultures as late as 36 hours after initiation of the response.
These results are in contrast to the response of B cells to red blood cell antigens (RBC). In the response to RBC, antigen alone is sufficient for initiation of the response, and only signal 2 is required thereafter. This nonspecific helper signal may be delivered by direct T cell-B cell interaction or by addition of Con A supernatant.

514 PHORBOL ESTER TRIGGERING OF THYMUS-DERIVED LYMPHOCYTES, Judith Pretell and Richard K. Gershon, Yale University School of Medicine, New Haven, CT 06510
The potent tumor promotor, 12-O-tetradecanoylphorbol-13-acetate (TPA), has been shown to exhibit marked effects on the immune function of murine lymphocytes. Populations of thymus-derived lymphocytes (T cells) cultured with TPA in vitro are greatly enhanced in their ability to give help to B cells in a plaque-forming response to sheep erythrocytes (SRBC). This help is only observed when the T cells are from mice primed with SRBC; naive T cells which have not received this antigenic signal cannot respond to the TPA stimulus and appear inhibited in their ability to give help to B cells. Such T cells cultured in vitro with SRBC and TPA simultaneously are also suppressed in their helper activity, while SRBC-immune T cells are enhanced. Although TPA is not mitogenic for naive T cells, it does increase DNA synthesis in SRBC-immune T cells within 24 hours of culture. The proliferative response of T cells activated polyclonally by concanavalin A (Con A) is not enhanced by co-culture with TPA during the first 48 hours; however, continued exposure to TPA results in enhanced DNA synthesis while that of cells activated by Con A alone is waning. Antisera treatment of these T cells with anti-Ly2 in order to eliminate $Ly1\text{-}23^+$ suppressor T cells leaves a population of cells enriched in $Ly1^+$ helper cells which give greater help to B cells than $Ly1^+$ cells obtained from T cells cultured with Con A alone. SRBC-immune T cells activated with Con A, TPA, or Con A and TPA all give considerably more help to B cells than do immune cells cultured alone. An analogy is suggested between the requirement for antigen as a primary signal and TPA as a secondary activation signal with that of two-stage carcinogenesis in which a carcinogen is required as an initiator and TPA acts as a promoting agent before carcinogenesis occurs.

515 MITOGEN INDUCED CHANGES IN HUMAN LYMPHOCYTE CALCIUM METABOLISM,
Andrew H. Lichtman, George B. Segel and Marshall A. Lichtman, University of Rochester School of Medicine, Rochester, NY 14642.
Calcium has been suggested as an internal second messenger when lymphocytes are stimulated by mitogens to enter the cell cycle. We have assessed the effect of two lymphocyte stimulants, the plant lectin phytohemagglutinin (PHA) and the calcium ionophore A23187, on human lymphocyte nucleic acid synthesis, total cell calcium content and ^{45}Ca labeling. We have used an ultrasensitive method for the measurement of total cell calcium in the same samples used for radiolabeling. Mitogenic concentrations of A23187 (~.25 µmol/L) caused an increase in both total cell calcium and ^{45}Ca labeling. These increases were almost completely blocked by inhibitors of mitochondrial respiration suggesting that the calcium increment after ionophore treatment was located in mitochondria In contrast, total cell calcium was not altered at optimal mitogenic PHA concentrations (0.1 µg/ml and above). However, at the minimum PHA concentrationsthat caused stimulation (0.025 to 0.1 µg/ml) the dose response of ^{45}Ca uptake was very similar to that of DNA synthesis. Importantly, we could not stimulate DNA synthesis with PHA without increasing lymphocyte ^{45}Ca labeling. Thus, an increase in total cell calcium is not essential for mitogenesis; however, an increase in ^{45}Ca exchange is closely associated with the mitogenic effects of A23187 and PHA.

516 T CELL DERIVED HELPER FACTOR BYPASSES THE THYMUS-REQUIREMENT DURING IN VIVO DIFFERENTIATION OF PRECURSOR T CELLS INTO CYTOTOXIC T CELLS . Klaus Pfizenmaier, Hermann Wagner and Martin Röllinghoff, Institute for medical microbiology, 65oo Mainz, W-Germany.

Athymic (nu/nu) mice lack immunocompetent mature Tcells. We here report, that prethymic lymphocytes from nu/nu mice contain alloreactive T cell precursors which differentiate in vivo into alloreactive CTL, provided Lyt 1 T cell-derived helper factor is applied together with the alloantigen. BALB/c nude mice were injected on two days with $2x1o^7$ irradiated C57Bl/6 spleen cells. Starting the same time, the nude mice received twice a day over a three day period 1oo ul of helperfactor (s.c. or iv.). At day 5, spleen and lymphnode cells were tested for cytotoxic activity. Specific lysis of $H-2^b$ target cell was obtained with splenic and lymphnode effector cells, which were sensitive to anti-Thy 1 plus complement treatment. This indicated, that the in vivo generated cytotoxic activity was mediated by nu/nu mouse derived Tcells. The results suggest, that Tcell precursors in athymic mice are already endowed with the V-gene repertoire required for alloreactivity, and that thymic processing is not mandatory for the activation of such precursor Tcells. T cell derived helper factor seems to substitute thymus function inasmuch as it triggers differentiation of immunoincompetent prethymic lymphocytes into specifically cytotoxic T cells.

517 PROSTAGLANDIN RELEASING FACTOR (PRF); PRODUCTION, CHARACTERISTICS, AND FUNCTION. Jerome A. Mattingly, Ohio State University, Department of Medical Microbiology, Columbus, Ohio 43210.

A factor released from Con A activated C57Bl/6 spleen cells causes the release of prostaglandins from macrophages. This "Prostaglandin Releasing Factor" (PRF) has been partially purified and characterized, as have the cells which produce it and the cells upon which it acts.

It appears that PRF is released from mitogen stimulated $Ly1^+2^+3^+$ splenic T-cells, as pretreatment of spleen cells with antisera to either Ly1 or Ly2, plus complement, abrogates the ability of spleen cells to produce this factor. Also, Con A stimulated cultures of $Ly1^+$ cells along with $Ly2^+$ cells fail to produce PRF.

Following passage through Sephadex G-200, the molecular weight of PRF is found to be 60-80 x 10^3 daltons. The activity of PRF has no H-2 restriction and appears to function in some xenogeneic systems. The target of PRF appears to be the macrophage. Immediately upon addition of PRF to macrophage cultures, these cells release prostaglandins into the medium; other cell cultures are negative for this aspect.

Attempts are presently being made to make antibodies against PRF. If these can be produced, then the fluorescein labelling of these antibodies will shed much light on PRF receptors, possible sub-populations of macrophages, and control of the immune response by macrophages. Also, anti-PRF might inactivate PRF in vivo, thereby decreasing states of immunological anergy associated with prostaglandins (aging, tumors).

518 ACTIVATION OF MURINE B LYMPHOCYTES BY MONOCLONAL ALLOANTIBODY TO sIgD, Ian M. Zitron, Jewish Hospital of St. Louis, St. Louis, MO 63110

The majority of murine splenic B cells bear both sIgM and sIgD. Evidence for a regulatory function for sIg has come from demonstrations that anti-Ig antibodies will induce at least some of these cells to proliferate. The anti-Ig antibodies used have either been polyvalent or anti-μ and no success has been reported using anti-δ reagents. I have generated and characterized a monoclonal somatic cell hybrid (68A2.C6) which produces alloanti-δ antibody. B cells from mice of the Ig^a and Ig^c allotype groups bear the target determinant. The Ig product of 68A2.C6 induces allotype-specific B cell proliferation; this is in contrast to another monoclonal anti-δ (10-4.22, Oi et al), which fails to do so. Cells which have been induced to proliferate by 68A2.C6 Ig show increased pfc responses to thymic-independent antigens upon reculture. Preliminary experiments indicate that this reflects an increase in precursor frequency.

This is the first demonstration of B cell triggering with a monoclonal product directed against an sIg determinant and the first direct demonstration of a triggering function for sIgD. As such, it provides an opportunity to examine in detail the intracellular changes and biological sequelae resulting from stimulation through sIgD.

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519 THE REQUIREMENT FOR TWO SIGNALS IN THE GENERATION OF THE Fc FRAGMENT INDUCED POLYCLONAL ANTIBODY RESPONSE, Edward L. Morgan, Marilyn L. Thoman and William O. Weigle, Scripps Clinic and Research Foundation, La Jolla, CA 92037

Fc fragments derived from human IgG1 induce murine B lymphocytes to undergo proliferation and differentiation to antibody secreting cells. The polyclonal antibody response requires both macrophages and T cells. The function of the macrophages is to cleave the Fc fragments into 14,000 molecular weight biologically active subfragments which then stimulate the B cells to proliferate. For the differentiation of these cells to polyclonal antibody production by Fc fragments, T cells are required. Spleen cell cultures from nude mice or T cell depleted normal mice proliferate but do not produce polyclonal antibody unless T cells are added. That the same B cell population responds to both the Fc signal and the T cell signal was shown by "hot thymidine pulse" experiments. The addition of high specific activity [^{3}H] thymidine to the cultures up to 48 hrs after initiation resulted in a drastic reduction in the polyclonal antibody response. The requirement for T cells can be substituted for by the addition of a soluble cell free factor(s). Purified T cell replacing factor (TRF) derived from Concanavalin A incubated spleen cells was used in conjunction with Fc fragments to produce a polyclonal antibody response in nude spleen cell cultures. The above data suggest that the Fc fragment induced polyclonal response occurs through two signals. One signal is associated with proliferation and is generated by the interaction of the mitogenic subfragment with the B cells. The second differentiation signal is derived from activated T cells or their soluble product. (Supported in part by USPHS AI07007)

520 UNIQUE SUPPRESSIVE EFFECT OF PHYSIOLOGICAL LEVELS OF CALCIUM ION ON THE SECONDARY IgG ANTIBODY RESPONSE IN VITRO, Sharyn M. Walker and William O. Weigle, Scripps Clinic and Research Foundation, La Jolla, CA 92037

The requirement for calcium ion (Ca^{++}) in various cellular metabolic activities, including those of lymphoid cells, is well appreciated. However, the amount of Ca^{++} necessary to support lymphoid cell expression is relatively small. This is shown, for example, by the Mishell-Dutton culture system in which a 10% serum supplement makes the medium about 0.1 mM in Ca^{++}, at least 10-fold less than the concentration of Ca^{++} in extracellular fluids. In the course of defining optimal culture conditions for generation of secondary IgG responses in vitro, it was found that the response of spleen cells from mice primed and boosted to TNP-KLH was routinely suppressed by as much as 100% when generated in medium containing physiological amounts of Ca^{++}. Suppression was entirely dependent on the concentration of TNP-KLH used to elicit antibody, with relatively high concentrations of antigen resulting in the greatest sensitivity to Ca^{++} level. The phase of the antibody response susceptible to Ca^{++} was localized to 24-48 hours after culture initiation. In contrast, polyclonal antibody responses were not affected by high Ca^{++} concentrations at any time. The mechanism whereby antigen dose and Ca^{++} concentration interact uniquely in controlling the IgG antibody response may be of significance not only in understanding regulation of lymphoid cell expression, but the role of cations in regulation of cell maturation and function in general. (Supported in part by USPHS grant AI07007 and American Cancer Society Junior Faculty Research Award, JFRA-19)

521 INHIBITION OF LYMPHOCYTE ACTIVATION BY AN ARACHIDONIC ACID HYDROPEROXIDE, Michael G. Goodman and William O. Weigle, Scripps Clinic and Research Foundation, La Jolla, CA 92037

Incubation of murine splenocytes with 15-hydroperoxyarachidonic acid generated from soybean lipoxidase-treated arachidonic acid (AA/L) profoundly inhibits the proliferation and maturation of these cells. This inhibition is not altered by addition of indomethacin, an inhibitor of prostaglandin synthetase, to cultures containing AA/L. AA/L interfered with RNA synthesis, protein synthesis, DNA synthesis, and blastogenesis, all with parallel inhibition profiles without adversely affecting lymphocyte viability. Several different lymphocyte subpopulations, distinguished by reactivity to different mitogens, were all rendered totally unresponsive to stimulation by addition of AA/L to culture. Furthermore, growth of the mastocytoma cell line, P-815, was arrested in the presence of the lipoxidase-catalyzed oxidation product of arachidonic acid, indicating that the mechanism of AA/L-mediated inhibition did not involve interference with receptor-ligand interaction. Proliferative and polyclonal responses to bacterial lipopolysaccharide were inhibited even when AA/L was added as late as 24 hrs after initiation of culture. Whereas a 10-30 min pre-incubation with AA/L at 37°C was sufficient to inhibit mitogenesis totally, pre-incubation at 4°C led only to partial inhibition, indicating that inhibition is, at least in part, an active process. The irreversible nature of the inhibitory activity mediated by AA/L was demonstrated in experiments in which this effect could not be reversed in the absence of AA/L in recovery periods up to 6 hrs before addition of LPS. (Supported in part by USPHS grants AI07007 and AI15284)

522 MODULATION OF DNA SYNTHESIS OF LYMPHOCYTES *in vitro* BY THE TUMOR PROMOTER, TPA
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The tumor promoter, 12-0-tetradecanoyl-13-phorbol acetate (TPA) can either enhance or depress DNA synthesis of bovine lymph node lymphocytes *in vitro* depending on the staging of the cell system. TPA added simultaneously with the mitogenic lectins ConA or PHA acts as a co-mitogen and increases the number of cells undergoing DNA synthesis (Mastro and Mueller, Exp. Cell Res. 88:40, 1974). In contrast, TPA at the same concentration (10^{-8}-10^{-7} M) blocks blast transformation and DNA synthesis of cells in mixed lymphocyte culture (Mastro and Mueller, Biochim. Biophys. Acta 517:246, 1978). This inhibition is reversible. Cells recover in fresh medium in the absence of TPA. Cells preincubated for 24 hrs in TPA give a depressed response in MLC and also a depressed response to ConA and PHA. Again the depression can be reversed. TPA is not metabolized significantly during the incubation period.

These findings that increased time of exposure to TPA either in MLC or by preincubation leads to a depressed mitogenic response, suggest a possible indirect effect of TPA. TPA may act by changing the plasma membrane recognition sites for the mixed lymphocyte response or for lectins; and/or by activating a subclass of cells to suppress the mitogenic response. We have preliminary data to suggest that the latter plays a role in the action of TPA in this system.

523 MURINE THYMIC LYMPHOMAGENESIS: MONOCLONAL ANTIBODY INDUCED LYMPHOMA CELL GROWTH ARREST,
M.S. McGrath, L. Jerabek, E. Pillemer, and I.L. Weissman, Stanford University School of Medicine, Stanford, CA 94305

We have approached the problem of thymic lymphomagenesis in mice from the point of view of cellular immunologists studying the maturation and antigen induced proliferation of thymocytes and their T cell progeny. We proposed a receptor mediated leukemogenesis hypothesis wherein T cell lymphomas would be clones of antigen recognizing T cells bearing surface receptors specific for recognition and response to the envelope glycoproteins of the inducing MuLV. We have shown: T cell lymphomas bear receptors which are specific for the inducing MuLV gp71 (leukemogenic, but not non-leukemogenic viruses); the presence of such receptors is a marker for irreversibly transformed leukemic cells and is not present on cells which predominate in the preleukemic period. To investigate the possibility that MuLV:lymphoma cell surface interactions are necessary for continued cellular proliferation , monoclonal antibodies recognizing AKR thymic lymphoma cell surface antigens were developed and used to inhibit in vitro lymphoma cell proliferation. Four antibodies to normal T cell surface antigens arrested T lymphoma cell proliferation at cell cycle phase G_1 ultimately leading to noncomplement mediated cell death within 48 hours whereas several MuLV and tumor specific antibodies showed no effect. This arrest was apparently due to blockade of MuLV:receptor interactions by the inhibitory antibodies and could only be prevented by preincubation of the cells with excess endogenously produced MuLV. Preincubation of lymphoma cells with excess MuLV does not alter the level of cell surface bound antibody for at least three of these antibodies, indicating that the most likely mechanism of inhibition is steric hindrance of specific viral:mitogen receptor interactions.

524 PROMOTION OF MURINE BONE MARROW STEM CELL DIFFERENTIATION *IN VITRO* BY ALLOGENEIC EFFECT FACTOR (AEF),
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Allogeneic effect factor (AEF), a short-term mixed lymphocyte culture supernatant of *in vivo* alloactivated T cells, is known to have profound effects on the differentiation of functional B and T lymphocytes *in vitro*. This study was designed to investigate the effects of AEF on cultures of murine bone marrow stem cells. Addition of AEF at low concentrations (0.1-1%) to fresh bone marrow cell cultures caused rapid growth of cells in these cultures for a period of several weeks as assessed by direct cell counts and uptake of tritiated thymidine. This effect was mediated in cultures supplemented with deficient horse serum which itself could not support stem cell differentiation, and, in contrast to Dexter-type cultures, did not require prior establishment of an adherent cell layer. Control cultures which were not supplemented with AEF, or cultures supplemented with various concentrations (2.5-25%) of a concanavalin A-stimulated lymphocyte supernatant possessing T cell growth factor (TCGF) activity, declined within 2-3 weeks and lost their CFU-S and CFU-C. Such CFU-S and CFU-C activity was maintained for a significantly longer period in the AEF-supplemented cultures. We have evidence indicating that the effects mediated by AEF were not due to contaminating colony stimulating factor (CSF). Furthermore, preliminary evidence suggests the development of lymphoid stem cells in these cultures.

525 MITOGEN-ENHANCED METABOLISM OF PHOSPHATIDYLINOSITOL IN LYMPHOCYTES: INHIBITION BY PLASMA LIPOPROTEINS, David Y. Hui and Judith A.K. Harmony, Indiana University, Bloomington, Indiana 47405

When challenged with mitogenic agents such as phytohemagglutinin (PHA), human peripheral lymphocytes become mitotically active. One membrane-associated phenomenon which occurs immediately following addition of PHA is an increased rate of turnover of phosphatidylinositol (PI). Low density lipoproteins(LDL), which inhibit calcium ion accumulation by PHA-activated lymphocytes, inhibit enhanced PI turnover. The extent of inhibition of PI metabolism by LDL depends on the concentration of LDL present in the incubation medium: 50% of maximum inhibition occurs at an LDL protein concentration of 33 μg/ml and maximum inhibition occurs at concentrations above 150 μg/ml. PHA stimulates ^{32}P incorporation into mono-, di-, and triphosphoinositide. However, LDL abolish ^{32}P incorporation into monophosphoinositide without affecting incorporation into di- and triphosphoinositide. The ability of LDL to inhibit PHA-induced PI turnover is mimicked by EGTA at concentrations sufficient to complex the extracellular calcium ion, thereby preventing PHA-enhanced accumulation of cellular Ca^{2+}. Furthermore, inhibition by LDL of PHA-induced monophosphoinositide turnover correlates directly with inhibition by LDL of calcium ion accumulation. These results suggest that enhanced calcium ion accumulation is required for PHA to elicit turnover of monophosphoinositide and that inhibition of this mitogen-induced response by LDL is a direct consequence of the ability of these lipoproteins to prevent calcium ion accumulation. The step in monophosphoinositide metabolism which is sensitive to LDL and, by inference, to calcium ion is release of phosphoinositol from monophosphoinositide.

Intracellular Events in Growth and Development

526 PROTEIN SYNTHESIS AND SECRETION DURING PANCREATIC DEVELOPMENT, Gary A. Van Nest and William J. Rutter, Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143.

Changes in protein synthesis and secretion associated with pancreatic development in the rat were analyzed by two dimensional gel electrophoresis. The embryonic pancreas selectively secretes two sets of proteins differing in tissue specificity. One set consists of the digestive (pro)enzymes secreted by the adult pancreas. The second set is a limited group of proteins that is not secreted by the adult pancreas but is secreted by embryonic gut, lung, and mesenchymal tissue. Almost all of the proteins detected in gels of total adult pancreatic proteins are secretory (pro)enzymes, demonstrating the overwhelming commitment of the adult pancreas to the synthesis of digestive (pro)enzymes. In contrast, the total proteins synthesized by embryonic pancreases up to 18 days gestation are vastly more complex than the secretory proteins. The digestive (pro)enzymes are not detected in gels of total pancreatic proteins until 17 days gestation. The total proteins of the embryonic pancreas change very little from 14 to 18 days gestation and are similar to the total proteins of embryonic gut, lung, and mesenchyme. It appears that differentiation of the pancreas involves changes in expression of a limited set of genes(primarily those coding for the digestive enzymes). Monoclonal antibodies that bind specifically to pancreatic membranes have been developed as probes to monitor the changes in cell surface proteins during pancreatic development. Analysis of 300 hybridoma cultures producing antibodies against pancreas membranes indicates that 39 are specific to pancreas. Supported by NIH grants AM21344 and AM05674-03.

527 UNIVALENT CATION CONCENTRATION AND REGULATION OF THE BALB/C-3T3 CELL GROWTH CYCLE. C.N. Frantz and C.D. Scher, Harvard Medical School, Boston, MA 02115

Addition of serum to density-arrested 3T3 cells causes a rapid increase in uptake of Na^+ and K^+, followed 12 hr later by the onset of DNA synthesis. Both K^+ influx and DNA synthesis are inhibited by ouabain. Rozengurt[1] has proposed that serum initiates cell growth by increasing the intracellular K^+ concentration $(K^+)_i$. We have examined the role of $(K^+)_i$ in growth control. Density inhibition of Balb/c-3T3 growth was associated with a marked decrease in both $(Na^+)_i$ and $(K^+)_i$. $(Na^+)_i$ and $(K^+)_i$ were 40 and 180mM in subconfluent cells, and 14 and 90mM in density-inhibited cells, respectively. Serum addition to density-inhibited cells caused an increase in $(Na^+)_i$ to 20mM within 1 hr, and an increase in $(K^+)_i$ to 107mM within 4 hrs. Density-inhibited cells were stimulated with serum in the presence or absence of 0.1mM ouabain; both groups of cells entered S phase at identical rates. Without ouabain, $(Na^+)_i$ increased to 20 and $(K^+)_i$ to 107mM. With ouabain, however, the $(K^+)_i$ decreased within 1 hr and fell to 62mM. Clearly, the serum-stimulated increase in $(K^+)_i$ does not regulate the entry of cells into the S phase. A high ouabain concentration (0.5mM) did not inhibit platelet factor-induced competence, but did inhibit later cell cycle events (progression) regulated by plasma. Inhibition of plasma-mediated events only occurred when the $(K^+)_i$ fell below 50mM and was correlated with a cessation of protein synthesis. Thus, increased $(K^+)_i$ does not regulate Balb/c-3T3 growth. Decreased $(K^+)_i$ inhibits cell growth by inhibiting protein synthesis.

[1]Rozengurt, E. Cold Spring Harbor Conferences on Cell Proliferation 6, 673 (1979).

528 The Isolation from Cartilage of an Inducer and an Inhibitor of Capillary Endothelial Cell Growth and Migration. M. Klagsbrun, R.S. Langer, C.J. Scheiner, J. Folkman and B.R. Zetter. Children's Hospital and Harvard Medical School, Boston, MA. 02115.
Cartilage is an avascular tissue that is invaded by blood vessels during endochondral bone development. We have identified factors in bovine scapular cartilage that are antagonistic in their effects on both proliferation and migration of bovine adrenal capillary endothelial cells. A polypeptide with a molecular weight of 16,300 and a pI between 9 and 10 has been isolated from bovine scapular cartilage and purified to homogeneity. Addition of this factor (1 μg/ml) to sparsely plated (500 cells/cm^2) capillary endothelial cell cultures caused a 13-fold increase in cell number in 7 days. Control cultures incubated in Dulbecco's modified Eagle's medium with 10% calf serum (DME-10cs) alone increased in cell number by less than 3-fold. Migration analysis was performed by measuring the area of phagokinetic tracks left by the endothelial cells as they moved across gold-coated coverslips. The mean phagokinetic track area for unstimulated cells in DME-10cs was 11,700 μm^2 after 18 hr. Addition of the cartilage-derived stimulator at an optimal concentration of 0.6 μg/ml increased the mean track area to 19,000 μm^2. Another factor has been isolated from the same cartilage and partially purified by affinity chromatography on sepharose-trypsin columns. At 500 μg/ml, this factor completely inhibits the stimulatory effects of the 16,300 M.W. factor on proliferation and migration. These results suggest that cartilage vascularization may be regulated by an interplay of cartilage-derived factors. An imbalance in this system may lead to abnormal cartilage vascularization as occurs in cartilage tumors and in arthritis.

529 PROLIFERATION OF ROUS SARCOMA VIRUS-INFECTED, BUT NOT OF NORMAL, CHICKEN FIBROBLASTS IN A MEDIUM OF REDUCED CALCIUM AND MAGNESIUM CONCENTRATION, Samuel D. Balk, University of Manitoba, Winnipeg, Canada R3E 0W3.
Both normal and Rous sarcoma virus-infected chicken fibroblasts proliferate actively in a culture medium containing physiological concentrations of calcium (1.2 mM) and magnesium (0.7 mM). In the presence of a physiological concentration of magnesium, reduction of the calcium concentration to 0.125 mM resulted in a significant decrease in the proliferation of the normal, but not of the neoplastic, fibroblasts. Reduction of the magnesium concentration to 0.05 mM in the presence of a physiological concentration of calcium had a similar effect. In a culture medium containing reduced concentrations of both calcium (0.20 mM) and magnesium (0.05 mM), the normal fibroblasts were maintained without proliferation, whereas the Rous sarcoma virus-infected fibroblasts continued to proliferate actively. The cytosol concentrations of ionized calcium and magnesium are known to be regulated by a balance between net passive influx and active extrusion and sequestration. On the basis of this consideration and the findings described above it can be hypothesized that: (i) Fibroblast replication is iniated when cytosolic concentrations of calcium,magnesium,or both rise above a critical level.(ii) Autonomous initiation of replication of neoplastic fibroblasts is a result of failure of cytoplasmic divalent cation homeostasis;alternatively,sarcoma virus infection may endow cells with a divalent cation-independent mechanism that bypasses an initiation mechanism that is,normally, divalent cation-dependent.(iii)Proliferation of normal fibroblasts is controlled by extracellular matrix components that interact with cell surfaces in a manner that limits the permeability of plasma membranes to divalent cations.

530 PATHWAYS IN THE INTERNALIZATION OF NERVE GROWTH FACTOR, Bruce A. Yankner and Eric M. Shooter, Dept. Neurobio., Stanford University School of Medicine, Stanford, Ca.94305.

The clonal cell line of rat PC12 pheochromocytoma responds to nerve growth factor (NGF) by the extension of neurite processes. During the first 4 to 6 hr of incubation with NGF, a rapidly dissociable binding site is selectively downregulated whereas a tightly bound component is maintained at steady state. Subsequently, the tightly bound component rises over a course of 6 days and parallels the time course of neurite outgrowth.
Previous work has demonstrated that NGF is translocated intact to the nucleus of the PC12 cell where it interacts with detergent-resistant receptors on the nuclear membrane(Yankner and Shooter, 1979 PNAS 76, 1269-1273). It was shown that cells in suspension accumulate NGF in the nucleus with a delay of 20 min. Cells in monolayer, however, exhibit a 3-4 hr delay. This difference correlates with a considerably greater cellular degradation of NGF in monolayer than in suspension. The addition of 100 μM chloroquine to cells in monolayer inhibits the release of TCA-soluble ^{125}I from cell bound ^{125}I-βNGF by about 80% and also reduces the delay in nuclear translocation to 30 min. At a chloroquine concentration as low as 10 μM, the NGF in the nucleus was increased by about 100% over control after a 24 hr incubation. In addition, the biological activity of NGF in terms of the percentage of neurite-bearing cells was also doubled. Pulse chase experiments suggest that the NGF translocated to the nucleus after 4 hr results from internalization after that time and not from the intracellular redistribution of hormone internalized earlier. During the first 4 to 6 hr of incubation, internalized NGF appears to be directed to the lysosomes as reflected in chloroquine-sensitive downregulation. Subsequently the degradative avenue having been saturated or partially inactivated, a new pathway to the nucleus appears. Correlations are observed between the association of NGF with the nucleus and the neurite outgrowth response.

534 MITOGENIC HORMONE-INDUCED INTRACELLULAR MESSAGE: ASSAY AND PARTIAL CHARACTERIZATION OF AN ACTIVATOR OF DNA REPLICATION INDUCED BY EPIDERMAL GROWTH FACTOR. Manjusri Das, Biochemistry & Biophysics, Univ. of Pennsylvania Sch. Med., Philadelphia, Pa. 19104

This work explores the pathway from nuclear quiescence to mitogenesis. It describes an *in vitro* assay for an activator of DNA replication induced by epidermal growth factor (EGF) in responsive cells. Cytoplasmic extracts from EGF-treated 3T3 cells were found to contain substances that can stimulate DNA synthesis in isolated nuclei from spleen cells of adult frogs. Extracts from untreated resting 3T3 cells lack this activity, and EGF itself is incapable of stimulating DNA synthesis in these cell-free systems. The extract-induced stimulation of incorporation of [^{3}H]dTTP into nuclear DNA is ATP dependent and requires the presence of the four deoxyribonucleoside triphosphates, suggesting the occurrence of replication rather than repair synthesis. This cell-free assay has been used to obtain some initial insights into the mechanism of induction and biochemical characterization of the intermediate in EGF action. Half-maximal induction of the active intracellular substance is achieved at about 0.08nM EGF, a concentration that correlates well with the concentration required for half-maximal mitogenesis. Studies on the biochemical characteristics of this active substance strongly suggest that the activity is associated with a protein. Sucrose gradient centrifugation of the extract revealed three peaks of activity with molecular weights of 46,000, 110,000 and 270,000 daltons. These results, together with my previous observations on receptor dynamics (Proc. Natl. Acad. Sci. 74: 2790; Ibid. 75: 2644, 1978) indicate that receptor-EGF interaction at the cell surface leads to the intracellular generation of proteins which are capable of stimulating quiescent nuclei into activity. (Supported by NIH grants AM 25819 and AM 25724)

535 EFFECTS OF VIRAL TRANSFORMATION OR TREATMENT WITH A TUMOR PROMOTER ON CHICK MYOTUBES IN VITRO, C. M. West, Y. Toyama, H. Holtzer & D. Boettiger, Departments of Anatomy and Microbiology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Myotubes were cultured from 11-day chick embryos and treated with the tumor promoter phorbol-12-myristate-13-acetate (PMA) at 10^{-7} M. Within 24 hours the rates of synthesis of several myotube-unique luxury proteins was significantly and selectively inhibited whereas the synthesis of two intermediate filament subunits was modestly stimulated. Inversely, the degradation of the luxury proteins was increased while that of intermediate filament subunits was inhibited. Within 48 hours the tubular morphology of myotubes was replaced by a flattened one interrupted with numerous long processes. Within 3-4 days of the first detectable effect of PMA on protein turnover, nearly all of the myotubes were devoid of previously accumulated myofibrils and contained dense clusters of intermediate filaments and sarcoplasmic reticulum as determined by electron microscopy. The cells retained their identity as myotubes only insofar as they remained multinucleated, did not reenter the cell cycle, retained disorganized clumps of sarcoplasmic reticulum, and reverted back to a normal condition when PMA treatment was discontinued. A non-promoter analogue was without effect. Myotubes were also prepared which contained a ts-mutant of Rous sarcoma virus and were maintained at the non-permissive temperature for transformation. When these cells were shifted to the permissive temperature, however, an effect on the synthesis of luxury proteins and intermediate filament subunits similar to that seen with PMA was observed. Clearly one effect of these two transformation-related agents is a reversible interruption of the expression of the terminally differentiated state. This effect was exerted in the absence of any detectable traversal of the cell cycle.

536 SEGREGATION OF CHROMOSOMAL PROTEINS DURING CELL REPLICATION, Elizabeth Fowler and Roderick M. Farb, University of North Carolina, Chapel Hill, NC 27514

The distribution of chromosomal proteins to daughter DNA molecules has been studied in Chinese Hamster Ovary cells through two cell replications. Cells are grown for several generations in [^{14}C]-lysine and thymidine, followed by one generation in the presence of [^{3}H]-lysine and 5-bromodeoxyuridine and a further generation in unlabeled amino acid and thymidine. This protocol produces equal amounts of unifilarly and unsubstituted DNA. Chromatin containing the two types of DNA and the full complement of chromosomal proteins are separated by sucrose gradient centrifugation after ultraviolet irradiation. In multiple experiments 5-22% of the labeled proteins are estimated to segregate with the appropriate DNA strand while the remaining proteins are randomly distributed to daughter chromatin. When monomer nucleosomes prepared from the same chromatin are separated by density banding in CsCl, no segregation is observed. Thus, over several generations the four core histones do not appear to segregate with the DNA strand synthesized at the same time; the segregating species appear to be nonhistone proteins.

531 A SPECIFIC TRANSLATIONAL CONTROL MECHANISM OPERATES DURING GROWTH OF ANIMAL CELLS.
Dean L. Engelhardt and Gloria T-Y. Lee, Columbia University, College of Physicians and Surgeons, New York 10032 and Virology Committee, University of Chicago, Chicago 60637.
Using a two-dimensional polyacrylamide gel analysis we have shown that the production of some abundant cellular proteins is modulated as Vero cells grow into the stationary phase. Translation of total polysomal messenger RNA or polysomal polyadenylated RNA in vitro reveals that, in many instances, the level of functional mRNA coding for these growth-modulated peptides is itself correspondingly modulated. Two classes of events lead to this growth modulation of translating mRNA levels: the modulation in the total cytoplasmic levels of some mRNAs and the alteration in the ratio of the amount in the polysome fraction to that in the nonpolysome fraction. In this latter instance both specific increases and decreases are observed. This observation constitutes a translational control mechanism whereby the translation of certain proteins is selectively promoted or repressed against a background of invariant protein synthesis. The mRNAs regulated in this manner have the property that the ratio of polysomal to nonpolysomal amounts deviates significantly from the norm of the total mRNA (measured either by translation in vitro or by measurement of amount of poly(A)) regardless of whether the cells are in the exponential phase or the stationary phase. In stationary phase cells, where a lower proportion of total mRNA is being translated, this shift of ratios, either by specifically promoting or repressing translation, may be due to the intrinsic capacity of the mRNA (or mRNP) to be recruited into the polysomes. In this model mRNAs with high capacity for recruitment are selectively translated while those with low capacity are selectively repressed through a process of competetive recruitment.

532 The $G_1 \rightarrow S$ PHASE TRANSITION IN CHICK FIBROBLASTS: BLOCKAGE WITH TRANSAMINASE INHIBITORS,
John Groelke and Harold Amos. Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Mass. 02115.
Inhibition of protein synthesis for 6-8 hr with cycloheximide (CH) prevents subsequent insulin or somatomedin-stimulated DNA synthesis in quiescent chick embryo fibroblasts (CEF). The addition of one of several α-keto acids (pyruvate, oxaloacetate, α-ketobutyrate, but not α-ketoglutarate) at 0.5-1 mM concentrations or L-alanine at 0.1 mM restores DNA synthesis in previously inhibited cells when combined with insulin. If, following CH treatment, CEF are exposed to insulin and pyruvate or L-alanine plus an inhibitor of transaminase reactions (e.g., cycloserine (CS), aminooxyacetate (AOA)), insulin-stimulated DNA synthesis is initiated in the presence of pyruvate but not L-alanine. Unexpectedly, CS and AOA were found at 0.1-2 mM concentrations to block insulin-stimulated DNA synthesis when combined with hormone in CEF not exposed to CH. The CS and AOA inhibition of DNA synthesis was overcome by adding pyruvate, oxaloacetate, or α-ketobutyrate at 4 mM with insulin to cells simultaneously exposed to the transaminase inhibitors. L-alanine at concentrations up to 25 mM and α-ketoglutarate were without effect. Neither CS nor AOA inhibited protein synthesis as measured by incorporation of ^{3}H-leucine into acid insoluble material. As with CH treatment, AOA significantly reduced lactate production by insulin-treated cultures.

533 AN ANALYSIS OF THE INTRINSIC AND EXTERNAL REGULATION OF EPITHELIAL DIFFERENTIATION,
Tung-Tien Sun, Thomas I. Doran and Alda Vidrich, Departments of Dermatology and of Cell Biology and Anatomy, The Johns Hopkins Medical School, Baltimore, Maryland 21205.
The relative roles which "intrinsic divergence" and "external modulation" may play in regulating the differentiated states of various adult stratified squamous epithelia have been analyzed. Epithelial cells from rabbit skin, cornea and esophagus were grown identically in the presence of feeder cells. Under these in vitro conditions, the three epithelia remained distinguishable, even though they had lost some of their in vivo characteristics of differentiation and become less distinctive. However, when the cultures were trypsinized and the suspended cells injected subcutaneously into athymic (nude) mice, all three epithelia faithfully regained their in vivo differentiated states, as judged by both morphological and histochemical criteria; thus the injected (cultured) epidermal cells formed a keratinized cyst, corneal epithelial cells formed a non-keratinized cyst, and esophageal epithelial cells formed a parakeratinized cyst. Hence the epithelia of skin, cornea and esophagus do not assume the same morphologic differentiation even when provided with identical in vitro and in vivo environments. Such results suggest that these three epithelia have diverged significantly from each other during development, and that intrinsic divergence plays a major role in regulating their in vivo differentiated states. On the other hand, the fact that epithelium cultivated from a given tissue could faithfully regain its in vivo differentiated states under appropriate environments demonstrates that, under certain conditons, external modulation may also play an important role in regulating epithelial differentiation.

537 PROTEINS ASSOCIATED WITH KIRSTEN SARCOMA VIRUS. David A. Scheinberg and Mette Strand, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The Kirsten sarcoma virus (KiSV) is a defective recombinant transforming virus. We have recently characterized a defective woolly leukemia virus pseudotype particle of KiSV containing KiSV RNA, reverse transcriptase, and the major core protein, p28, of the helper woolly leukemia virus. Instead of the usual complement of helper virus proteins, these defective particles include three polypeptides previously not identified in RNA tumor viruses. We have analyzed these proteins to determine whether they were of KiSV, endogenous virus or cellular origin. Two of the proteins, of 110,000 and 55,000 daltons, were glycoproteins. The third, of 20,000 daltons, was a phosphoprotein, as was the 110,000 dalton protein. None of these proteins were immunologically related to known virus structural proteins, but remarkably, immunoprecipitation using several anti-virus sera indicated that the glycoproteins were found in a variety of rodent viruses. The properties of the 55,000 dalton protein suggested that it might be an endogenous virus envelope glycoprotein. In contrast, the 20,000 dalton phosphoprotein was specific to KiSV and therefore probably related to the KiSV sarcoma gene product. All three proteins could be detected in normal rat kidney cells, but what was more interesting was that upon transformation of these cells by KiSV, we found distinct alterations in these proteins: the 110,000 and 20,000 dalton protein became phosphorylated and appeared both in the cell plasma membrane and in the media as excreted proteins. The changes in the 110,000 dalton protein were observed not only after KiSV transformation, but also in cells transformed by DNA virus and chemicals as well. This protein may thus serve as a marker for fibroblast transformation.

538 MODULATION OF THE DIFFERENTIATION OF 3T3-L2 FIBROBLASTS INTO ADIPOCYTES BY VITAMIN A, Thomas Murray and Thomas R. Russell, University of Miami School of Medicine, Miami, Florida, 33101

Vitamin A is known to be necessary for the support of normal growth and development. It has also been shown to influence cellular differentiation, particularly the maintenance of certain differentiated epithelial cells. In light of its reported role in differentiation, vitamin A has been tested for its influence on the conversion of 3T3-L2 fibroblasts into adipose cells.

Cytodifferentiation of 3T3-L2 cells results in the acquisition of many biochemical, morphological, and hormonal properties characteristic of adipocytes. The degree to which cultures convert can be quantified in several ways, including measurement of lipogenic enzyme activity and microscopic monitoring of fat cell cluster development. This differentiation process is known to be promoted by pulsing confluent cultures of fibroblasts with 0.5mM 1-methyl-3-isobutylxanthine (MIX) and 0.25μM dexamethasone (DEX) for 48 to 72 hr. Vitamin A (10^{-6} to 10^{-8} M) can effectively inhibit the action of the promoting agents on the differentiation when administered with the promoters. The increase in fatty acid synthetase activity (FAS) (already known to be due to new enzyme synthesis) normally seen with MIX-DEX treatment is reduced by over 60% with 10^{-6}M vitamin A. This concentration of the vitamin also completely blocks the effects of the triggering agents on microscopically detectable adipocyte cluster development. Vitamin A does not appear to be toxic over the range of concentrations tested and its effects can be reversed. Cultures initially inhibited by vitamin A during MIX-DEX triggering will exhibit normal increases in FAS in response to a second pulse of MIX-DEX without vitamin A.

539 QUANTITATION OF TUBULIN POOLS IN FERTILIZED SEA URCHIN EGGS, C.Bruce Godfrey, Vincent D. Lee, and Leslie Wilson, University of California, Santa Barbara, Ca. 93106.

To understand the regulation of microtubule (MT) assembly and function we have characterized the colchicine (CLC) binding activity in S. purpuratus eggs and zygotes through the first mitotic cycle. Decay corrected CLC binding assays were employed for the quantitation of total and polymerized tubulin (Tu) pools. The K_d for the Tu-CLC binding reaction was invariant upon fertilization and through cleavage at 1.2 ± 0.2 uM. First order CLC binding decay half times in homogenates incubated at 37°C increased from 1.9 hrs to 4.2 hrs upon fertilization and remained constant thereafter. The total cellular Tu concentration was found to be 2.8 mg/ml at all times in the cell cycle. Egg or zygote MTs were collected by 200,000 g centrifugation of MT stabilizing buffer (SB) homogenates. The Tu-CLC binding activity in cold solublized supernatant fractions of SB pellets represents the polymerized Tu pool. CLC binding in these fractions was temperature sensitive, competitivly inhibited by podophyllotoxin (PLN), and showed a K_d of 1.7 uM. First order decay half times in polymerized Tu fractions were 5.5 ± 0.3 hrs and did not vary with fertilization or early development. We find that 10% ± 4% of the total Tu pool in unfertilized eggs behaves as though it were polymerized. Zygotes cultured in the presence of 1uM PLN, 10 uM vinblastine or griseofulvin, or exposed to 0°C for 20 min, showed 4%, 5%, 8% and 4% of their respective total Tu in the polymerized fraction. By comparison, polymerized Tu in parallel control cultures homogenized prior to cleavage reached maximum levels of 21% ± 3%, then fell to 17% ± 3% after cleavage. Exposure of zygotes to 50% D_2O sea water during metaphase reversibly enhanced polymerized Tu by 20%.Supported by ACS #CD-3E

540 ALTERATIONS IN cAMP-DEPENDENT PROTEIN KINASE ISOZYME PATTERNS AND POLYAMINE BIOSYNTHESIS IN S49 LYMPHOMA CELLS EXPOSED TO BT_2CAMP

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Cyclic AMP has been shown to be the second messenger for trophic hormone responses. Its action includes activation of cAMP-dependent protein kinases which mediate increases in general and/or specific protein and RNA synthesis. Abnormally high cyclic AMP levels result in the arrest of S49 lymphoma cells in the G_1 phase of the cell cycle. Concomitant with this induced G_1 cell cycle block Bt_2cAMP results in decreased activities of ornithine decarboxylase and s-adenosyl methionine decarboxylase. Exposure to Bt_2cAMP alters the relative amounts of cyclic AMP dependent protein kinase isozymes. Type I isozyme follows control levels in the non-treated cells, whereas type II isozyme activity totally disappeared. This laboratory has shown a similar response in Bt_2cAMP treated CHO cells, RAT-1 cells and RSV-RAT-1 cells. In the S49 lymphoma cells the type I isozyme was not activated to levels higher than control levels as shown in the other cell lines. These studies suggest that whereas endogenously generated cyclic AMP is positive for the stimulation of polyamine biosynthesis and cell proliferation the addition of exogenous cyclic AMP analogs which alter isozyme patterns exerts an inhibitory effect on the same events.

541 CHARACTERIZATION OF A PROTEIN MEDIATING ERYTHROPOIETIN INDUCED NUCLEAR TRANSCRIPTION.

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One of the earliest effects exerted by erythropoietin (epo) involed in the induction of erythrocyte differentiation, is stimulation of marrow cell transcription. Pure endotoxin-free, epo stimulates transcription in both rat and mouse marrow cell cultures. Hemoglobin synthesis is also stimulated by pure epo, free of endotoxin, in rat marrow cell cultures. There is evidence suggesting that epo interacts at the cell surface, however it is not known whether epo is internalized. Epo does not exert a direct effect upon transcription in isolated marrow nuclei. We have described a cytoplasmic factor (MCF) derived from epo-treated marrow cell which stimulates transcription in isolated nuclei[1,2]. The appearance of this marrow cytoplasmic protein factor (MCF) is not dependent upon prior protein synthesis and is induced by purified erythropoietin free of endotoxin. The MCF derived from rat bone marrow cultures exposed to endotoxin free epo can stimulate marrow nuclear transcription up to 130%. Both the rate of transcription and its extent are augmented by MCF. Evidence indicates that MCF can be fraction of molecular weight greater than 130,000. MCF can be pelleted through a 2M sucrose, 4mM $CaCl_2$ discontinuous gradient when centrifuged at 105,000xg for 24 hours. Preliminary evidence indicates that MCF can stimulate transcription by isolated marrow chromatin.

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Coated Pits and Vesicles in Intercellular Protein Transport

542 "Dansylcadaverine Inhibits Internalization of ^{125}I-Epidermal Growth Factor in Balb 3T3 Cells,"

Haigler, H.T., Maxfield, F.R., Willingham, M.C., and Pastan, I., National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205.

The binding and internalization of ^{125}I-epidermal growth factor (^{125}I-EGF) was studied in cultures of Balb 3T3 cells using a novel method that involved removal of cell-surface hormone by treatment with acetic acid under conditions that did not remove internalized hormone. In control cultures ^{125}I-EGF initially bound to its receptor on the plasma membrane and then was rapidly internalized. After 30 min, only 15% of the cell-bound hormone remained on the surface. In contrast, cultures treated with dansylcadaverine retained 82% of the cell-bound hormone on the cell surface. We propose that dansylcadaverine inhibits EGF internalization by preventing it from clustering in clathrin-coated pits.

543 DIFFERENTIATION-RELATED CHANGES IN PROCESSING OF HUMAN CHORIONIC GONADORRUPIN BOUND TO TARGET CELLS, A. Rees Midgley, Jr., Kenneth L. Campbell and Thomas D. Landefeld, University of Michigan, Ann Arbor, MI 48109

Human chorionic gonadotropin (hCG, 36700 daltons) binds to receptor sites on granulosa cells and causes these cells to differentiate into luteal cells. To determine if the hCG involved in initiating luteinization is processed differently from that which binds to luteal cells, we labeled isolated alpha and beta subunits of hCG with ^{131}I or ^{125}I. The labeled subunits were recombined with unlabeled subunits and the resulting recombined hCG was separated from free subunits by SDS electrophoresis. The SDS was removed from the recovered labeled hCG by substitution with Triton X-100 followed by absorption with Bio-Beads SM-2. The binding properties of the resulting preparations of labeled hCG were found to be almost indistinguishable from each other and from hCG labeled as an intact molecule. Mixtures of hCG labeled with different radioisotopes in the alpha and beta subunits were injected intravenously into hormonally primed rats containing high concentrations of receptor in either granulosa cells or in luteal cells. In contrast to non-target cells, granulosa cells and luteal cells concentrated each type of labeled hCG in a sedimentable fraction. However, a preferential loss of the radioactivity associated with alpha subunits was observed uniquely from granulosa cells. The resulting shift in ratios of bound radioactivity did not derive from changes in the circulating labeled hormones. Whether or not this unique processing is causally related to the ensuing luteinization of the granulosa cells remains to be determined.

544 CLATHRIN COATED VESICLES MEDIATE UPTAKE OF CELL SURFACE IMMUNOGLOBULIN IN A PROCESS THAT INVOLVES MICROFILAMENTS AND CALMODULIN, J.L. Salisbury, J.S. Condeelis and P. Satir, Anatomy, Albert Einstein College of Medicine, Bronx, New York 10461.

Cell surface IgM molecules of the cultured human lymphoblastoid cell line WiL2 are endocytosed into clathrin coated pits and coated vesicles after treatment with multivalent anti-IgM antibodies. Anti IgM receptors appear at first diffuse and not over coated regions of the membrane prior to challenge. Clustering of the complexes precedes association with coated regions of the plasma membrane. Studies with HRP indicate that total cellular endocytosis is inhibited by cold, azide, cytochalasin B,D and dihydrocytochalasin B (dCB) and stelazine but colchicine has no apparent affect on uptake. In EM studies of distribution of labeled IgM, dCB and stelazine both inhibit the association of labeled ligand clusters with coated regions of the membrane and their subsequent uptake by coated vesicles.Actin filaments decorated with HMM can be seen in direct association with coated pits and coated vesicles. Coated vesicles isolated from WiL2 cells retain significant levels of radiolabeled ligand. SDS PAGE reveals the major polypeptides of isolated coated vesicles to be a 180kd component that comigrates with calf brain clathrin and 42kd component that comigrates with rabbit muscle actin. Minor components include 110,70,55,36,30kd components and a 17kd polypeptide that comigrates with rat testis calmodulin. These observations indicate that cell surface receptor IgM is internalized by clathrin coated vesicles by a process which involves actin microfilaments and calmodulin. Supported by grants ACS BC-302 to JSC and USPHS HL 22560 to PS.

Plasma Components in Growth and Development

545 SPECIFIC REQUIREMENT FOR EPIDERMAL GROWTH FACTOR IN DEVELOPMENT OF CULTURED MAMMARY GLAND, Quentin J. Tonelli and Sam Sorof, Institute for Cancer Research, Phila.PA 19111

The mouse mammary gland in serum-free whole organ culture can be manipulated hormonally to undergo one complete cycle of lobuloalveolar development, functional differentiation, and regression, mimicking processes that occur in vivo. A second cycle has previously not been achieved in vitro. In search of chemical regulator(s) of development of mammary gland, we have identified a specific requirement for epidermal growth factor (EGF) in the morphological development of mammary lobuloalveoli. EGF acting in concert with insulin, prolactin, aldosterone and hydrocortisone, enables the mouse mammary gland to undergo a second development from the fully regressed state in culture. The same four hormones without EGF, other polypeptide growth factors, serum, and other hormones are all ineffective. The activity of EGF is maximal at physiological concentration (10 nM). At least three waves of DNA synthesis appear to be involved. The mouse mammary gland can thus now be induced in culture to undergo two complete cycles of mammary gland development and regression.

Chemical carcinogens are able to transform the mammary glands in whole organ culture, but analogous noncarcinogens do not. The transformed glands, which are dysplastic, metaplastic and oncogenic, have escaped the growth controls normally imposed by prolactin and specific steroids. The finding of the role of EGF in mammary gland development, together with a number of associations of EGF with transformation and tumorigenicity in other systems, raise the question whether a deregulation in EGF function mediates the loss of controls of organ development that characterizes the mammary gland transformation by chemical carcinogens.

546 PURIFICATION AND CHARACTERIZATION OF SOMATOMEDIN-BINDING PROTEIN PRODUCED BY RAT LIVER CELLS, D.J. Knauer and G.L. Smith, University of Nebraska, Lincoln, Ne, 68588
Multiplication - stimulating activity(MSA) consists of a family of low molecular weight polypeptides purified from serum-free culture medium conditioned by a cloned line of rat liver cells(BRL-3A). The MSA polypeptides appear to be structurally and functionally analogous to the human somatomedins. It has recently been demonstrated that the MSA polypeptides in BRL conditioned medium exist primarily in a high molecular weight form, as the result of specific association with a 25,000 dalton somatomedin-binding protein also produced by BRL-3A cells in culture(Moses et al.,1979).
In this report we demonstrate the purification of rat somatomedin-binding protein produced by BRL-3A cells in culture. The binding protein was purified from the complex mixture of proteins in BRL-3A conditioned medium by Dowex-50 ion exchange chromatography, Sephadex G-50 chromatography, and affinity chromatography utilising an affinity column of MSA polypeptides covalently coupled to Sepharose 4B. Data on the partial biophysical and biochemical characterization of the binding protein will also be presented.
In addition to the above studies, cultures of BRL-3A cells were grown in the presence of ^{3}H-glucosamine and ^{14}C-amino acids in order to further characterize the biochemical nature of the binding protein. The results of these experiments indicate that rat somatomedin-binding protein is a glycoprotein, although the extent of glycosylation and the nature of the carbohydrate moiety have not yet been determined.

547 SOMATOMEDIN C PERMITS PROGRESSION OF BALB/C-3T3 CELLS THROUGH G_1/S BOUNDARY, W.J. Pledger and Walker Wharton, University of North Carolina, Chapel Hill, N.C. 27514
Methylglyoxal bis-(guanyl hydrazone) (mGAG), an inhibitor of S-adenosyl methonine decarboxylase and polyamine synthesis, in concentrations of 20-100μM blocked the stimulation of DNA synthesis in quiescent Balb/c-3T3 cells treated with platelet derived growth factor (PDGF) and platelet-poor plasma (PPP). Competence formation produced by a transient (6 hr) exposure of quiescent cells to PDGF was not inhibited by mGAG; in contrast, mGAG effectively inhibited G_1 progression of competent cells in PPP, although maximal inhibition was observed when mGAG was present during both competence and progression. Even though it was necessary for mGAG to be present 0-4 hrs after addition of the mitogens to produce a maximal effect, the termination point of this metabolic block was 4 hr prior to S phase. This inhibition in cell cycle traverse was apparently due to reversible inhibition of protein synthesis. Removal of mGAG after a 12-18 hr exposure to the inhibitor with PDGF and PPP allowed the cells to enter S phase with first order kinetics after a 4 hr lag. The rate of entry into S, but not the time necessary for the cells to progress from the mGAG block into S, was dependent on the concentration of PPP after removal of the mGAG. Exogenous spermine did not alter this 4 hr lag time before entry into S. Media supplemented with either somatomedin C (10^{-10}-10^{-9}M) or insulin (10^{-7}-10^{-6}M) was able to substitute for platelet-poor plasma to allow commitment to DNA synthesis and entry into S phase. (Supported by NIH Grant CA24193)

548 MULTIPLICATION STIMULATING ACTIVITY (MSA) FROM THE BRL 3A RAT LIVER CELL LINE: RELATION TO HUMAN SOMATOMEDINS AND INSULIN, Matthew M. Rechler, George S. King, Ellen E. Schilling and S. Peter Nissley, National Institutes of Health, Bethesda, MD 20205

MSA, one of the insulin-like growth factors or somatomedins, possesses a similar spectrum of biological activities to insulin. This results from chemical similarities and from cross-reaction with specific receptors for the other polypeptide. We have used MSA purified from the BRL 3A rat liver cell line, and pork insulin, to dissect whether the common biological responses are mediated via insulin receptors, MSA receptors, or both. In some of these studies, Fab fragments prepared from antibodies to insulin receptors have been used to selectively block insulin receptors. The Fab fragments inhibit the stimulation of glucose oxidation in rat adipocytes by both insulin and MSA, demonstrating that both hormones act via the insulin receptor. The Fab fragments do not inhibit the stimulation of DNA synthesis by MSA and insulin in cultured human fibroblasts indicating that a receptor other than the insulin receptor (possibly the MSA receptor) mediates the mitogenic effects of both peptides. In HTC rat hepatoma cells, MSA, insulin and proinsulin appear to stimulate tyrosine aminotransferase and α-aminoisobutyric acid transport via both the MSA and insulin receptors.

Both MSA and insulin may have important roles in fetal growth. Fibroblasts from an infant with leprechaunism, low birth weight and insulin resistance have a selective absence of insulin receptors, presumably a primary genetic defect. MSA is synthesized by explants of fetal rat liver, and MSA levels are 100-fold higher in fetal rat serum than in maternal serum.

549 CHARACTERIZATION OF AN ENDOTHELIAL CELL DERIVED GROWTH FACTOR, Paul E. DiCorleto, Corinne Gajdusek, Stephen M. Schwartz, and Russell Ross, University of Washington School of Medicine, Seattle, WA 98195

Bovine aortic endothelial cells in culture release into serum free media or media containing platelet-free plasma derived serum a polypeptide that stimulates the growth of several cell types including 3T3, smooth muscle, and human dermal fibroblasts. The mitogenic activity is sensitive to trypsin and heat (80°C for 3 minutes), and exhibits an apparent molecular weight of 10-30,000 daltons by gel filtration in 1N acetic acid. The endothelial cell derived growth factor (ECDGF) appears distinct from the platelet derived growth factor (PDGF) by its heat lability and by different behavior on ion exchange chromatography (CM-Sephadex and DEAE-Sephadex). Also, the ratio of ECDGF to PDGF stimulation varies greatly in different cell types tested. ECDGF also appears distinct from epidermal growth factor (EGF) in that the former is capable of stimulating DNA synthesis in a 3T3 variant that is non-responsive to and lacks receptors for EGF. ECDGF is also not inhibited by antiserum to EGF. Submaximal doses of ECDGF and fetal calf serum act in an additive manner in the stimulation of DNA synthesis by confluent 3T3 cells.

550 CHEMICAL AND BIOLOGICAL PROPERTIES OF PLATELET DERIVED GROWTH FACTOR Å. Wasteson, C.-H. Heldin and B. Westermark Uppsala University, S-751 23 Uppsala, Sweden

The purification of a basic platelet-derived growth factor from outdated human platelets by a procedure involving ion exchange chromatography, hydrophobic chromatography, gel chromatography and gel electrophoresis in SDS was recently described (P.N.A.S 76(1979) 3722-3726). The gel chromatography step has now been improved, resulting in highly purified PDGF without the use of preparative gel electrophoresis in SDS. The product migrated like a 30,000 dalton protein in analytical gel electrophoresis in SDS; after reduction it gave rise to two distinct stainable bands, about 17,000 and 14,000 daltons, respectively, supporting the two-chain molecular model for PDGF suggested previously. Amino acid analysis indicated as expected a high percentage of basic amino acids. After labelling of lysine residues with 125-I according to the Bolton-Hunter procedure the radioactivity was approx. equally distributed between the two constituent polypeptide chains, as revealed by analytical gel electrophoresis in SDS. In contrast tyrosine residues available for labelling according to the chloramine-T method were predominantly located in one of the polypeptide chains. Antibodies were raised against PDGF in rabbit. IgG prepared from immune serum, but not from control serum, removed the biological activity(stimulatory activity on DNA synthesis in glial cells) from a solution containing PDGF. The precipitation of 125-I labelled PDGF with antibody was utilized to develop a radioimmunoassay for PDGF.

551 ABOLITION OF DNA SYNTHESIS AND MITOSIS IN FROG LENS EPITHELIUM BY HYPOPHYSECTOMY AND RESTORATION BY SOMATOMEDIN-C, Judson J. Van Wyk, University of North Carolina School of Medicine, Chapel Hill, NC 27514 and Howard Rothstein, Kresge Eye Institute of Wayne State University, Detroit, MI 48201

Hypophysectomy of frogs is followed 3-4 weeks later by total cessation of DNA synthesis and mitosis in lens epithelium due to arrest in G_0. Proliferation of these cells *in vivo* is restored by administration of growth hormone (GH), homologous prolactin, or TSH. Cultured frog lens from hypox frogs do not respond to these agents or to T_3 *in vitro*, but DNA synthesis is restored by 20% normal frog serum. Serum from hypox frogs, however, is not mitogenic for this tissue. By heterologous RIA and receptor assays, the content of somatomedin-like activity is diminished in frog serum following hypophysectomy but rises to above normal control levels by injections of either GH or T_3. Injection of essentially pure somatomedin-C into hypox frogs restores nuclear labeling with ^{3}H-thymidine. This is the first demonstration of an *in vivo* effect on cellular proliferation by a pure somatomedin and suggests that the mitogenic effects of the pituitary hormones are mediated through the induction of somatomedin-like peptides.

552 REGULATION OF KIDNEY EPITHELIAL CELLS GROWTH BY HORMONES, Mary Taub, Ben U, Lorraine Chuman, Milton H. Saier, Jr., and Gordon Sato, UC San Diego, La Jolla, Ca. 92093

We are investigation the possibility that (a) the supplements required for the growth of the Madin Darby Canine Kidney (MDCK) cell line in serum free medium are indeed requirements for the growth of normal kidney cells in vitro, and (b) that alterations in these growth requirements occur upon malignant transformation. MDCK cells grow at equivalent rates in Medium K-1 (serum-free medium supplemented with insulin, transferrin, T_3, hydrocortisone, and PGE_1) and in serum supplemented medium. Consistent with the hypothesis that MDCK cells resemble normal kidney cells in culture, we observed that primary cultures of baby mouse kidney epithelial cells grow in Medium K-1, and respond to the 5 components in the medium. MDCK cells do not form tumors in adult nude mice. We examined the possibility that changes in growth requirements in vitro occurred concommittantly with the acquisition of tumorigenicity by MDCK cells. Variants of MDCK cells have been isolated, which have lost the PGE_1 requirement for growth in defined medium. These variant cells are tumorigenic in adult nude mice. This observation suggests that los of a hormone growth requirement (in particular PGE_1) causes MDCK cells to become tumorigenic. The growth properties of Maloney Sarcoma Virus (MSV) transformed MDCK cells in defined medium have also been examined. Although these cells form tumors in adult nude mice, they still respond to the 5 factors in Medium K-1. However, unlike MDCK cells, the MSV transformants require CIG coated dishes and a relatively high inoculation density in order to grow in Medium K-1.

553 GROWTH AND DIFFERENTIATION OF RAT MAMMARY CELLS IN CULTURE: EFFECTS OF HORMONES IN SERUM-FREE CONDITIONS, J.E. Errick, J. Huisentruit, and T. Kano-Sueoka, Department of Molecular, Cellular and Developmental Biology, Univ. of Colorado, Boulder, CO 80309.

The development of the mammary gland in vivo is completely under hormonal control, and in its differentiated state, the mammary gland produces large amounts of characteristic proteins and fats. For these reasons, it is a good model system for the study of hormonal mechanisms. Primary cultures of mammary glands from mid-pregnant or estrogen-primed female rats have been shown to respond dramatically to insulin (I), hydrocortisone (H), and prolactin (P), as measured by ^{3}H-thymidine incorporation studies. While H and P alone or together have no effect, the combinations IP, IH, and IHP result in stimulation far above that in I alone. These effects can be seen in medium 199 with or without 1% fetal calf serum. Furthermore, addition of a recently identified growth factor, phosphoethanolamine (Kano-Sueoka et al., 1979, PNAS, in press), to medium 199 plus 1% fetal calf serum enhances the effects of I, IH, IP, and IHP approximately twofold. These cells in primary culture contain large amounts of lipid, as detected by Oil Red O staining and the modulation of the synthesis of this lipid by the above-mentioned hormones and others is being investigated through the use of ^{14}C-acetate incorporation into lipids. Finally, a cell line, 22-1, isolated from a hormone-independent rat mammary tumor has been shown to synthesize and store large amounts of fat in the presence of fetal calf serum and not in calf serum. A serum-free medium for growth of these cells is being developed, and so far, we have observed fat-containing cells in these serum-free cultures. We are studying the roles of hormones such as I, H, and P in the proliferation and differentiation of the 22-1 cells.

554 HORMONAL EFFECTS ON THE STRUCTURE AND FUNCTION OF THYROID CELLS IN CULTURE. B. Westermark, K. Westermark and J.A. Snyder, The Wallenberg Laboratory, Uppsala Univ. Sweden, and MCD Biology, University of Colorado, Boulder, CO 80309, USA.

Thyroid cells in primary culture retain most of their differentiated functions and continue to produce hormone when exposed to thyrotropin (TSH). Serially propagated thyroid cells do not synthesize hormone but retain a TSH sensitive adenylate cyclase. On such partially dedifferentiated cells, TSH induces a gross change in cell morphology, ultimately leading to a total arborization. Using time lapse cinematography, immunofluorescence with actin antibodies and high voltage EM, we have shown that the TSH induced arborization is preceded by a total loss of actin containing microfilament bundles. This effect is caused by the increase in cellular cyclic AMP induced by TSH; arborization can also be induced by dibutyryl cyclic AMP and is enhanced by phosphodiesterase inhibitors. Since several other hormone responsive cells react in a similar way to second messenger cAMP, we conclude that cyclic AMP may be a major promoter of microfilament bundle disassembly.

In contrast to the general assumption that TSH is a stimulator of thyroid growth in vivo, TSH is not a growth factor for cultured thyroid cells. High doses of TSH as well as dibutyryl cyclic AMP and phosphodiesterase inhibitors are in fact growth inhibitory. Epidermal growth factor (EGF) stimulates the growth of sheep follicular cells when added at low serum concentration whereas high serum concentration stimulates the growth of both epithelial cells and stromal fibroblasts. We have thus found a medium (MEM, 1-3% FCS, 10 ng EGF/ml) that sustains the growth of thyroid follicular cells with a minimal growth of fibroblasts. (Supported by NIH 5P41 RR000 592, EMBO and the Swedish Cancer Society).

555 GROWTH AND MAINTENANCE OF HUMAN EPITHELIAL CELLS, Edwin V. Gaffney, Margaret A. Grimaldi, The Pennsylvania State University, University Park, Pa. 16802.
The ectoderm-derived amnion membrane can be enzymatically dissociated to a single cell suspension to establish pure epithelial-like primary cultures. We have investigated the effect on amnion cell growth of three human blood-derived nutritional supplements: whole blood serum (WBS), platelet poor defibrinogenated plasma (plasma), and plasma derived serum (PDS). Cell growth is regulated by two factors: one released during platelet mediated intrinsic coagulation and another lost during the formation of serum from plasma through extrinsic clotting. Proliferation equivalent to that in WBS was observed when EGF or FGF were assayed with plasma or PDS in combination with hydrocortisone. Hydrocortisone alone slightly enhanced growth in the presence of PDS but not in the presence of plasma. In contrast, insulin lacked growth stimulating activity. Dialyzed plasma or PDS permitted a response to growth factors during the first 10 days of culture, but cell loss occurred thereafter. The results show that cell maintenance was restored by a small molecular weight factor recovered from the dialysates.

556 DIFFERENTIATION OF MOUSE EMBRYO CELLS INDUCED BY PLASMA. Bruce R. Krawisz, Dagne L. Florine, C. Garrison Fathman and Robert E. Scott, Mayo Clinic, Rochester, MN 55901.
Foci of differentiated cells formed when a variety of mouse embryo cell lines were cultured in medium containing 30% human plasma. These cell lines included Balb/3T3 cells, Balb/3T3 T proadipocytes, methylcholanthrene and Simian virus 40 transformed Balb/3T3 cells and C3H 10T½ and methylcholanthrene transformed 10T½ cells. Differentiated cells contained numerous cytoplasmic granules which by electron microscopy were found to be bound by a unit membrane; undifferentiated cells did not. Differentiated cells contained numerous phagocytic or autophagic vacuoles and residual bodies and they contained abundant acid phosphatase activity; undifferentiated cells did not. Differentiated cells also stained with an antisera against Ia cell surface antigens; undifferentiated cells did not. Growth of mouse embryo cells in high concentrations of plasma therefore promotes differentiation into macrophage-like cells. As in other studies we found that differentiated cells were growth arrested in the G_1 phase of the cell cycle by autoradiographic and flow microfluorimetric analysis. Since differantiation was not observed when cells were cultured in serum, declotted plasma or in heat inactivated plasma we propose that differentiation in this system may be induced by a specific plasma factor(s). These data suggest that mouse embryo cells are multipotential stem cells which can differentiate not only into adipocytes, chondrocytes, rhabdomyocytes, vascular pericytes and fibroblasts, as we and others have recently reported, but also into macrophage-like cells. The identification and isolation of the specific factor(s) which induce cellular differentiation should be of great value in studies on the mechanisms for control of the reciprocal coupling of growth and differentiation.

557 EVIDENCE FOR A LOW-MOLECULAR-WEIGHT INSULIN-LIKE PLASMA PEPTIDE WHICH STIMULATES CHICK CHONDROCYTE METABOLISM, Loren Pickart, Virginia Mason Research Center, Seattle , WA 98101
Plasma contains a number of insulin-like activities (ILA) or somatomedins of 3 to 6 thousand d which may function as growth factors. We have found evidence for a smaller insulin-like activity (SILA) of 800 d which markedly stimulates the metabolism of chick and, to a lesser extent, rat chondrocytes.
SILA was extracted from human Cohn fraction IV-1 by procedures similar to those used for somatomedin isolations. At the G-75 column separation step, the 300-1,000 d fraction was found to possess ILA on chondrocytes. Rechromatography on G-25 concentrated activity in 3 peptides of about 800 d. An HPLC separation on RSil C-18 RP (10 micron) gave elution of the active peptide at 18% acetonitrile in water. SILA appears to be a hydrophobic peptide which is free of lipids, carbohydrates,nucleic acids, metal ions, and immunoreactive insulin. Unlike somatomedins A and C, SILA did not displace insulin from placental membranes. SILA markedly increased the metabolism of cultured chick chondrocytes, but was only 30% as active on rat chondrocytes. When added at 1 ug/ml to chick chondrocytes cultured in F-12 medium plus 1.5% fetal calf serum, SILA increased DNA synthesis 7.3-fold, lipid synthesis 10.2-fold, and lactate production 2.9-fold after 48 hr incubation. These results suggest that low-molecular-weight peptides, which are smaller than the somatomedins, may contribute to the total ILA of human plasma. (Supported by CA27129).

558 INHIBITION OF FIBROBLASTIC ADENYLATE CYCLASE ACTIVITY BY A PARTIALLY PURIFIED SERUM FACTOR, Wayne B. Anderson and Cynthia J. Jaworski, National Cancer Institute, Bethesda, MD 20205

The intracellular concentration of cyclic AMP appears to influence many aspects of cell behavior, including growth rate and differentiation. Recently, we have shown that the growth promoters MSA and EGF significantly inhibit the hormone-stimulated accumulation of cyclic AMP in intact cells. Serum also antagonizes the stimulatory effects of hormones on the adenylate cyclase activity of intact cells. A factor has now been partially purified from calf serum which selectively inhibits the GTP and hormone-stimulated cyclase activity of plasma membrane preparations; basal activity and fluoride-stimulated activity are only marginally altered. Initially, this inhibitory component is isolated as a 60,000 MW protein complex by sephadex fractionation and DEAE cellulose chromatography. A low MW inhibitory factor can be extracted from the 60,000 MW species with acetone-ethanol and further purified by LH 20 column chromatography. The inhibitory effect elicited by the serum factor is both time and temperature dependent. Initial experiments indicate that phosphorylation of plasma membrane proteins is enhanced in the presence of serum factor. This suggests that the mechanism of action of the factor in inhibiting hormonal responsiveness may be related to the phosphorylation of certain membrane components.

Density-Dependent Growth Control

559 FOREIGN BODY TUMORIGENESIS: CLONAL ESCAPE FROM DENSITY DEPENDENT GROWTH CONTROL (DDGC) IN VIVO, Charles W. Boone and Robert Scott, National Cancer Institute, Bethesda, MD, 20205, and Mayo Clinic, Rochester, MN, 55901.

In vivo a foreign body stimulates fibroblasts to proliferate and form a capsule that tends to wall it off under conditions of DDGC. In close analogy, *in vitro* the tissue culture dish stimulates fibroblasts to attempt the same thing by forming a multilayered "capsule" that also exhibits DDGC. Similarly, when Balb/3T3 or C3H/10T 1/2 cells are implanted subcutaneously in syngeneic mice attached to a 0.2x5x10 mm plastic plate, tumors develop after 4 months that possess unique tumor associated transplantation antigens and distinct karyotypes, indicating that they are also derived from single cells, and that also demonstrate loss of density, anchorage, and serum growth controls. Exposure of the plate-attached cells to 5-azacytidine or the promotor phorbol myristate acetate accelerates the development of tumors to a degree that is dose dependent, a fact that has formed the basis of a novel bioassay for carcinogens and tumor promotors. Of additional biological significance is the finding that histological sections of tumors developing from plate-attached Balb/3T3 cells showed foci of differentiation into various neoplastic mesenchymal cell patterns including malignant hemangiopericytoma from the capillary pericye, malignant hemangioendothelioma from the endothelial cell, chondrosarcoma from the chondrocyte, granulocytic sarcoma from the myeloblast, and fibrosarcoma from the fibroblast.

560 GROWTH CONTROL OF SIMIAN VIRUS 40 TRANSFORMED CELLS BY COCULTIVATION ON NORMAL MONOLAYERS, Christine C. Robinson and John M. Lehman, University of Colorado Health Sciences Center, Denver, Colorado 80262

Chinese hamster embryo cells transformed by temperature sensitive SV_{40} T antigen mutants (tsA mutants) at low temperature, return to a more "normal" phenotype at high temperature, suggesting that T antigen maintains some aspects of transformation. These cells at high temperature are not completely normal, however, for they do not arrest in G_1 (G_0) when confluent on plastic.

In contrast, when the tsA mutant virus transformed cells are cocultivated at high temperature on monolayers of normal, confluent cells they will arrest in G_1. The ability of the tsA transformants to enter a G_0 resting state upon cocultivation is under T antigen control; it does not occur with wild-type virus transformed cells at high or low temperature or with tsA transformed cells at low temperature. These results suggest that T antigen, rather than functioning as a direct initiation of cell DNA synthesis, may control the potential of transformed cells to respond to regulation by normal cells. The use of this isogenetic system, in which the response to cocultivation can be manipulated simply by temperature, will be used to study the nature of the regulation provided by the normal cells and the changes in the transformed cells which render them sensitive or insensitive to such factors.

561 EPITHELIAL CELLS MIGRATE BY BEING PUSHED, Philip Rosen and Dayton S. Misfeldt, Stanford University School of Medicine and Veterans Admin. Hospital, Palo Alto, CA 94305

The dog kidney epithelial cell line (MDCK) shows a density correlated inhibition of growth. When a confluent monolayer is 'wounded' cells migrate as a sheet into the wound area and proliferate to their previous density (~6.5×10^5 cells/cm^2). Precise measurements of the rate of migration over five days revealed that the cells accelerated at a constant rate of 0.26μm.hr^{-2} with an apparent initial velocity of 3.2μm.hr^{-1} at the time of wounding. This was interpreted as arising from each cell exerting a pressure on its neighbours. Therefore the resultant net force at the wound edge would be density dependent. To verify this wounds were made at submaximum cell densities. In these experiments the cells did not migrate until a minimum density of 2.3×10^5 cells/cm^2 was reached. At that density the cell sheet began to accelerate at the previous rate. It is concluded that epithelial cells migrate as a sheet by being pushed.

Hematopoietic Stem Cells

562 FETAL HEMOGLOBIN IN CLONES DERIVED FROM PLURIPOTENT HEMOPOIETIC PROGENITORS (CFU-GEMM), BFU-E & CFU-E by H.A. Messner and A.A. Fauser, Institute of Medical Science, Ontario Cancer Institute, University of Toronto, Canada.

Clonal assays for CFU-GEMM, BFU-E and CFU-E permit the identification of progeny derived from precursors that differ in their level of erythroid differentiation, and facilitate the assessment of differentiation related properties. In extension of reports by Papayannopoulou et al. (PNAS 1976;77) we have examined clones grown from CFU-GEMM, BFU-E and CFU-E for their content of HbF by immunofluorescence or radioimmune assay. The erythroid component of more than 90% of all mixed hemopoietic colonies derived from CFU-GEMM contained HbF. The frequency was significantly lower for erythroid bursts and further diminished for erythroid colonies. Quantitative assessment by radioimmune assay yielded HbF concentrations for erythroid colonies between 0 and 500 pg. The amounts of HbF in erythroid bursts varied between 0 and 5,000 pg. The measured amount of HbF did not correlate with the size of the examined bursts. The addition of medium conditioned by leukocytes in the presence of phytohemagglutinin (PHA-LCM) increased the frequency of HbF containing bursts without influencing the quantitative distribution of HbF. In conclusion, these results are consistent with a view that more primitive erythroid progenitors are more likely to give rise to HbF containing progeny. The similarity in the distributions of HbF concentrations suggest a regulatory mechanism responsive for HbF synthesis that is intrinsic to the progenitor cells.

563 EXPRESSION OF RED CELL MEMBRANE PROTEINS IN ERYTHROID CELLS. Peter Yurchenco and Heinz Furthmayr, Yale University, New Haven, CT. 06510.

Specific antibodies to human glycophorin A and spectrin were used to study the expression of these membrane proteins in normal and pathologic human bone marrow. In immunofluorescence experiments spectrin and glycophorin A are found in 50-60% of the nucleated cells in normal bone marrow. These two proteins are expressed at all stages of red cell differentiation and can be traced at least to the earliest morphologically recognizable nucleated red cell precursor, the proerythroblast; the two proteins are specific for cells of the red cell series and are not found to be expressed in lymphocytic, granulocytic cells or platelets. These conclusions were drawn from studies on bone marrow in patients with a temporary block in erythropoiesis at the level of stem cells or of the proerythroblast. Bone marrow from these individuals either lacks all nucleated cells stainable for glycophorin A and spectrin or contains only proerythroblasts. Similar findings were obtained on spleen cells from mice which were made severely anemic by multiple injections with N-acetyl-phenylhydrazine. Antibodies to a sialoglycoprotein isolated from mouse red cell membranes stain 70-80% of all cells in the spleen of anemic animals, while only 1-2% of such cells are seen in the spleen of normal animals. Spectrin and glycophorin A could be labeled metabolically and isolated using specific antibodies. The human tumor cell line K562 expresses both membrane proteins, but induction experiments with various agents thus far have failed to change their expression.

564 A MONOCLONAL ANTIBODY THAT RECOGNIZES PRE-B CELLS AND A SUBSET OF B CELLS IN MOUSE,
Robert L. Coffman and Irving L. Weissman, Stanford University, Stanford, Ca. 94305

We have made several monoclonal antibodies which are specific for mouse bone marrow lymphocytes and for B but not T cells in peripheral tissues. These antibodies were produced by fusing mouse myeloma cells with spleen cells from rats immunized with the Ableson leukemia virus-induced cell line RAW 112. One of these antibodies, RA3-2C2, recognizes a surface antigen expressed on 30% of adult bone marrow cells, 20% of spleen cells and 12% of lymph node cells, but is not detected on thymocytes. About 1/3 of the RA3-2C2^{+} bone marrow cells are surface immunoglobulin positive (sIg^{+}) B cells, 1/3 are sIg^{-} small lymphocytes and the remaining 1/3 are sIg^{-} medium and large lymphocytes. After 2 days of <u>in vitro</u> culture, 5 to 10% of sIg^{-} bone marrow cells will express sIg but up to 50% of the small RA3-2C2^{+}, Ig^{-} bone marrow cells will express sIg, therefore this fraction is substantially enriched for at least one type of pre-B cell. In the spleen, all RA3-2C2^{+} cells are sIg^{+}, but only 30-40% of the sIg^{+} cells are RA3-2C2^{+}. Thus, RA3-2C2 appears to be a very useful antibody for the isolation of pre-B cells as well as for the study of B cell maturation.

565 HUMORAL REGULATION OF MURINE PLURIPOTENT HEMOPOIETIC STEM CELL DIFFERENTIATION.
Emilia Frindel and Dominique Dumenil (INSERM-U66). Institut Gustave Roussy, Villejuif

The mechanism of the choice of the pluripotent hemopoietic stem cell (CFU-S) to differentiate toward one of the cell lineages is not well known though some suggestions have been made that specific humoral factors act directly on these cells (G. van Zant and E. Goldwasser, Science <u>198</u>, 733, 1977). We present data demonstrating the existence of diffusible factors secreted by treated bone marrow capable of influencing the differentiation pathways of CFU-S. After Ara-C treatment, these factors orient CFU-S differentiation toward erythropoiesis, after RX irradiation to granulopoiesis (H. Croizat and E. Frindel, submitted for publication), and after antigen treatment, to granulo and megakaryocytopoiesis (F. Lepault and E. Frindel, in preparation). Data is presented showing that the factors act at the CFU-S level and not on the already committed stem cells : anti-CFU treatment abolishes the formation of spleen colonies but maintains the numbers of CFC in these bone marrows. Moreover, there is a competitive orientation toward the cell lineages : an increase in the E/G ratios of spleen colonies, an increase of BFU_E and a decrease of CFC in the case of incubation with factors secreted by Ara C treated marrow. It therefore seems possible to postulate that differentiation pathways of CFU-S can be modulated by diffusible long range factors and that the microenvironment may play a rôle in the expression of differentiation but not necessarily in its determination. The cells responsible for the secretion of the regulatory factors, the modification of the responder cell membrane receptors as well as the biochemical nature of these substances are under study using an in vivo-in vitro technique. The correlation with factors controlling proliferation is being considered.

566 SPONTANEOUS REGRESSION OF RFV INDUCED LEUKEMIA: ROLE OF INFECTION OF THE PLURIPOTENT HEMATOPOIETIC STEM CELL,
Candace S. Johnson, John Marcelletti, and Philip Furmanski, Michigan Cancer Foundation, Detroit, MI 48201

Mice infected with the RFV strain of Friend virus develop an erythroleukemia that spontaneously regresses in about 50% of the leukemic mice. We have shown that bone marrow CFU-c's in all leukemic mice become infected by 15 days post virus-inoculation. In regressor leukemic mice, the infected CFU-c's are eliminated and replaced by uninfected precursor cells that give rise to uninfected, functionally normal, mature macrophages which are involved in causing regression of the disease. In progressor mice, infected CFU-c's are not eliminated and mature into infected, functionally inhibited macrophages which are incapable of causing disease regression. To determine whether virus infection of CFU-s's might occur in progressor mice and thus preclude repopulation of the CFU-c compartment with uninfected cells, we tested bone marrow CFU-s's from leukemic mice for susceptibility to cytotoxicity by monospecific anti-viral-gp70 antiserum. Specific cytotoxicity was assayed by reduction in spleen colony formation in irradiated mice following exposure of the bone marrow cells to antiserum and complement. No specific cytotoxicity of CFU-s's was observed in bone marrows from progressor or regressor leukemic mice even when 50% of the total bone marrow population was killed (determined by trypan blue exclusion). In the presence of nonspecific rabbit anti-mouse serum and complement, spleen colony formation was reduced in direct proportion to cytotoxicity of the total population. We conclude that bone marrow CFU-s's do not become productively infected with virus during erythroleukemogenesis. (Supported by grants CA 14100 and CA 06419 and an institutional grant from the United Foundation of Detroit.)

567 CHROMOSOME MARKER EVIDENCE FOR BIPOTENTIALITY OF BFU-E, David L. McLeod, Mona L. Shreeve and Arthur A. Axelrad, University of Toronto, Toronto, Canada, M5S 1A8

Murine bone marrow or spleen cells seeded in plasma or agar cultures containing high concentrations of erythropoietin (epo) with or without poke-weed conditioned medium (PWCM) give rise to megakaryocyte colonies and erythropoietic bursts that are detectable by day 5 and reach maximum size by day 7. More than 40% of the erythropoietic bursts can be seen to contain megakaryocytes; these have been named mega-erythro bursts. Indirect evidence from this laboratory has supported the hypothesis that progenitors of erythropoietic bursts are bipotential and capable of giving rise to both megakaryocytes and cells of the erythrocytic series./ In the present study the Y chromosome, distinguished by the absence of distinctly staining centromeric heterochromatin, was used as a chromosome marker to determine whether or not cells in mega-erythro bursts were clonal. Male and female bone marrow cells from adult C57BL/6 mice were mixed in different proportions and seeded in agar cultures containing epo and PWCM. A total of 239 metaphases from 27 bursts were analysed. Metaphases within each burst were found to be either male or female but not both; tetraploid metaphases (considered to be megakaryocytic) and diploid metaphases (considered to be erythropoietic) within the same burst were of the same sex. These results are consistent with what might be expected if each mega-erythro burst were produced by a single progenitor cell and provide direct evidence for the bipotentiality of BFU-E.

568 HETEROGENEITY IN HEMOPOIETIC STEM CELL SELF-RENEWAL DURING COLONY FORMATION IN VITRO. R.K.Humphries, A.C.Eaves and C.J.Eaves, B.C. Cancer Research Centre, Vancouver, B.C.
We have recently shown that mouse hemopoietic stem cells plated in methyl cellulose cultures containing PWM spleen cell conditioned medium and erythropoietin produce colonies that achieve macroscopic size and contain erythrocytes, megakaryocytes, granulocytes, and a variety of progenitor cell types including CFU-S (Nature 279:718, 1979; Exp. Hematol. Today, 1980 in press). Such cultures have now been used to analyze the self-renewal behaviour of individual hemopoietic stem cells proliferating in vitro. Colonies derived from pluripotent stem cells were generated by plating 2-5 x 10^4 two week flask cultured cells in 1 ml methyl cellulose cultures containing PWM spleen cell conditioned medium and erythropoietin. Nine days later they could be distinguished from colonies derived from CFU-C and BFU-E by their content of erythroblasts (red colour) and megakaryocytes (large cells), and by their macroscopic size (0.2-9×10^5 nucleated cells/colony). Fifty such colonies were individually replated in secondary assays. Twenty-nine of these produced macroscopic colonies at least as large ($.7$-10×10^5 nucleated cells/colony) that contained megakaryocytes as well as erythroid cells. Continuing self-renewal during the formation of secondary colonies was demonstrable in 3 of 20 such colonies tested in tertiary assays. Variation in the stem cell content of primary colonies (range = 0-36, S.D. = 9.1) was significantly greater ($p < .001$) than that expected from sampling error alone, and self-renewal was not correlated with primary colony size. These results are similar to data on the extent of CFU-S self renewal in individual spleen colonies previously obtained by others. The present studies indicate that heterogeneity in stem cell self-renewal persists under conditions where stimulation by soluble factors excludes microenvironmental influences.

569 LEUKEMIA VIRUS INFECTIONS OF MOUSE HEMATOPOIETIC CELL CULTURES, Natalie M. Teich and T. Michael Dexter, The Imperial Cancer Research Fund, Lincoln's Inn Fields, London, WC2A 3PX, and The Paterson Laboratories, Wilmslow Road, Manchester, M20 9BX, England.

Mouse bone marrow cultures can be maintained for long periods in vitro and support the proliferation of the pluripotential hematopoietic stem cell (CFU-S), granulopoiesis and, with appropriate conditions, erythropoiesis and lymphopoiesis. Infection of such cultures with murine retroviruses has led to the establishment of permanent cell lines of different hematopoietic lineages. Our studies on infection with the Friend erythroleukemia virus complex have resulted in cell lines displaying the following phenotypic characteristics: (1) myelocytic leukemia lines which differentiate spontaneously in vitro and in vivo; (2) myelomonocytic leukemia lines which differentiate spontaneously in vitro and in vivo; (3) "null" cell lines of blast morphology which do not differentiate or induce leukemias; (4) a nonleukemic blast cell line which functionally resembles the committed granulocyte precursor cell (CFU-C), and (5) a nonleukemic blast cell line which differentiates along the erythroid, granulocytic and megakaryocytic pathways in vivo and thus could be classified as a tripotential or restricted CFU-S. The availability of these lines provides an opportunity to analyze the differences between leukemic and nonleukemic cells in regard to histochemical and immunological markers, responses to physiological and nonphysiological regulators of differentiation and other parameters of biological function.

570 HEMATOPOIETIC DIFFERENTIATION ANTIGENS IDENTIFIED BY ANTISERA AGAINST MOUSE BRAIN,
Joan W. Berman and Ross S. Basch, Pathology, N.Y.U. Medical Center, N.Y., N.Y. 10016

Rabbit antisera to mouse brain contain antibodies which are cytotoxic for multipotential stem cells (CFU-s). This activity persists after extensive absorption with liver, red blood cells and thymocytes. Although the absorbed antiserum kills fewer than 5% of the cells of the bone marrow and virtually no spleen cells, it has not proved to be a useful fluorescent reagent for the detection of stem cells, since it contains other antibodies which, though not cytocidal, are detectable by immunofluorescence. The major contaminating specificity is of considerable interest, since it reacts primarily with mononuclear phagocytic cells and immature granulocytes. This activity can be greatly reduced by absorption with resident peritoneal macrophages. Alternatively, cells bearing this specificity can be isolated from bone marrow suspensions by immunoaffinity techniques using a serum depleted of its anti-CFU-s activity. As a by-product of this procedure, we have produced a population significantly enriched in CFU-s. Using these techniques, we have examined the distribution of cells bearing these antigens in several strains of mice, under a variety of physiologic conditions. The CFU-s antigen resists tryptic digestion. It is present on a subset of thymocytes. These are numerous in young animals and appear to be the earliest cells in the intra-thymic developmental sequence. They decline with advancing age, but reappear in the thymus of preleukemic mice. As expected, the antigen present on mononuclear phagocytes is widely distributed in the organs of the reticulo-endothelial system.

571 Proliferation of HSC in the absence of an adherent monolayer. Christine Eastment, Elizabeth Denholm, Irena Katsnelson, Eugene Arnold, and Paul O.P. Ts'o. Johns Hopkins University, Baltimore, Maryland 21205.

Recent experiments on long term liquid cultures of murine bone marrow (BM) indicate that the production of hematopoietic stem cells (HSC) is dependent upon the establishment of an adherent monolayer. In studies on BM cultures derived from the Syrian Golden hamster, we have found that while an adherent layer may be essential for initial production of HSC, these HSC will continue to proliferate and differentiate in the absence of a monolayer for periods in excess of 6 weeks. These cells are morphologically similar to the putative HSC which have been described in murine systems and appear as an undifferentiated "blast" form. The "blast" cells will reconstitute lethally irradiated hamsters using the standard CFU-S assay. Colonies containing erythroid, myeloid, monocytic and megakaryotic elements are produced in soft agar cultures of pokeweed mitogen stimulated spleen conditioned medium (PSCM). Addition of PSCM to liquid cultures results in increased cell proliferation, appearance of differentiated forms, and the generation of an adherent monolayer similar to that seen in parent cultures. Removal of PSCM results in the disappearance of differentiated forms and the subsequent reappearance of the "blast" cell type. This system provides a means for identification of regulatory factors which stimulate of inhibit HSC.

572 COLONY FORMATION IN-VITRO BY MURINE MULTIPOTENTIAL HEMOPOIETIC CELLS, by G.R. Johnson, Institute of Medical Science, Ontario Cancer Institute, University of Toronto, Canada.

Previous studies have demonstrated that single cells obtained from fetal liver are capable of producing clones containing in addition to erythroid cells, variable numbers of neutrophils, macrophages, eosinophils and megakaryocytes. These mixed-erythroid colonies develop in the presence of a factor(s) present in pokeweed-mitogen stimulated spleen cell conditioned medium (SCM). Similar colony types have also been obtained from adult spleen, peripheral blood and bone marrow. With bone marrow cultures 40-50 erythroid colonies develop per 10^5 cells cultured, of which 40%-50% contain other hemopoietic populations. The adult erythroid colony-forming cells were mainly non-cycling (12.5% reduction in colony numbers after incubation with H^3-thymidine or hydroxyurea). Bone marrow erythroid colony forming cells sedimented with a peak of 4.5 mm/hr compared with CFU-S which sedimented at 4.25 mm/hr. The addition of erythropoietin (up to 4 units) to cultures containing SCM did not alter the number or degree of hemoglobinization of erythroid colonies. The effect of erythropoietin addition was strain dependent having little effect upon erythroid colonies obtained from CBA mice but causing an increase in the number of erythroid colonies obtained from C57BL/6 bone marrow or fetal liver cells stimulated with SCM. These data were obtained using either the standard agar cultures or the serum free methylcellulose culture system developed by Iscove.

573 PLURIPOTENT HEMOPOIETIC PROGENITORS IN POLYCYTHEMIA RUBRA VERA: ASSESSMENT IN CELL CULTURE, by A.A. Fauser and H.A. Messner, Institute of Medical Science, Ontario Cancer Institute, University of Toronto, Canada.

Human pluripotent hemopoietic progenitors (CFU-GEMM) give rise to mixed colonies that contain more than 1 lineage of hemopoietic differentiation when cultured with medium conditioned by leukocytes in the presence of phytohemagglutinin (PHA-LCM) and erythropoietin (EPO). We have used the assay to examine typical properties of CFU-GEMM in Polycythemia vera (PRV) as a model for human stem cell disorders: that is, we studied the EPO requirement and assessed their proliferative state. In newly diagnosed untreated patients with PRV and patients controlled by phlebotomy, mixed colonies with erythroid components were identified in cultures without exogenously added EPO. The addition of EPO usually yielded an increase in the number of mixed hemopoietic colonies. The proliferative state of CFU-GEMM that form mixed colonies without added EPO was examined using the H^3TdR suicide technique. Brief exposure to H^3TdR yielded a reduced plating efficiency of mixed colonies 20 to 35%. This observation differs from cell cycle studies in normal individuals where CFU-GEMM are found to be quiescent under steady state conditions.

In conclusion, CFU-GEMM were observed in all examined patients with PRV. In contrast to normal individuals, some CFU-GEMM give rise to erythroid progeny without the addition of EPO, thus sharing a typical property previously described for BFU-E & CFU-E. The cell cycle data indicate an increase in the proliferative activity of CFU-GEMM suggesting an increased growth rate as possible mechanism for PRV.

574 ENDOTHELIAL REGULATION OF MYELOID DIFFERENTIATION, Peter J. Quesenberry and Michael Gimbrone, Peter Bent Brigham Hospital and Harvard Medical School, Boston, MA and University of Virginia School of Medicine, Charlottesville, VA 22908.

Primary cultures of human endothelial cells (HEC) produce colony-stimulating activity (CSA) and basal production is stimulated by endotoxin or granulocyte lysates. In the present study HEC cultured for 24-48 hours with Hypaque-Ficoll separated human blood granulocytes (1.8-2.5 x 10^6/ml) lead to increased supernatant CSA levels. To further characterize the influence of granulocyte products on HEC CSA production, lysates were prepared by repeatedly freeze-thawing 16 x 10^6/ml non-adherent, dense (1.070 gm/cm^3) human blood cells (90-99% granulocytes) and their effects compared with lactoferrin (LF), a granulocyte component which inhibits CSA production in monocytes, and transferrin (TF) an inactive iron-binding protein. Conditioned media from HEC incubated with control medium, 10^{-7}M TF, 10^{-7}M LF, and 1% lysate stimulated 57 ± 16, 21 ± 1, 28 ± 12, and 155 ± 15 (mean±SEM) granulocyte-monocyte colonies (CFU-C)/10^5 marrow cells/10^6 HEC, respectively. In addition, freshly isolated HEC were incorporated into agar underlayers and their CFU-C stimulation compared to that of Hypaque-Ficoll separated human blood monocytes. In 7-14 day cultures, 10^5-10^6 HEC/ml stimulated 2-5 times more CFU-C than comparable numbers of monocytes. Media from indomethacin (1 ug/ml) treated HEC showed decreased CFU-C inhibitor levels with an increase in apparent CSA. These data indicate that HEC are a potent source of CSA; prostaglandins may be involved in modulating HEC-CSA production; and a granulocyte component other than lactoferrin can stimulate HEC-CSA production. The role of vascular endothelium as a physiologic regulator of granulopoiesis deserves further study.

575 LONG-TERM CULTURE OF MURINE BONE MARROW _IN VITRO_ REVEALS SIGNIFICANT GENOTYPIC VARIATION IN LONGEVITY OF HEMATOPOIESIS, Joel S. Greenberger and Maryann Sakakeeny, Joint Center for Radiation Therapy, Department of Radiation Therapy, and Sidney Farber Cancer Institute, and Harvard Medical School, Boston, Mass. 02115.

Culture of mouse bone marrow in 25% horse or fetal calf serum supplemented with 10^{-7}M hydrocortisone results in continuous proliferation of granulocyte-macrophage progenitor cells (CFUc) and hemopoietic stem cells (CFUs). No additional marrow is added and all nonadherent cells are removed each week at time of medium change. In experiments with inbred mouse strains, 3 distinct groups were detected with respect to longevity of granulopoiesis. Marrow cultures established from NZB, HRS, 129/J, C3H/HeJ, or SJL/J mice (Group I) failed to generate granulocytes past 25 weeks or CFUc beyond 20 weeks. In marked contrast, marrow from C57Ks/J, C57Br/J, NZW, DBA2/J, or AKR/J (Group II) generated granulocytes for over 50 weeks and CFUc for over 40 weeks. Other mouse strains tested including: Balb c/J, C57 Leaden/J, C57BL6/J, C57B110 Sn/J, NIH Swiss, C58/J, CBA/J, and Rf/J, (Group III); were intermediate between the other groups. To determine the genetic transmission of Group II growth, F1 hybrid mice of Group I and II parents were tested. (C3HXDBA/2) F1 marrow demonstrated growth characteristics of the (Group I) C3H/HeJ parent. In contrast, (C57Ks/J x NIH Swiss) F1 marrow demonstrated longevity of the (Group II) C57Ks/J parent. Studies with F2 hybrid and backcross generations of C57Ks/J and NIH Swiss demonstrated that longevity in long-term marrow cultures segregated as a genetic characteristic. Mixing stroma of one strain with hemopoietic stem cells of another may permit identification of genetic loci which affect the interaction of stromal and hematopoietic stem cells _in vitro_.

Teratocarcinomas

576 CELL LINES DERIVED FROM A T/+ TESTICULAR TERATOCARCINOMA, Helena R. Axelrod, Gerald B. Dooher, Dorothea Bennett, Sloan-Kettering Institute for Cancer Research, New York, N.Y. 10021

Genes in the T/t-complex of the mouse play an important role during embryonic development. To study their effect at the cellular and biochemical level, it would be useful to have embryonic cell lines derived from mice which carry mutations in the T/t-complex. Three types of continuous cell lines have been isolated from a spontaneous testicular teratocarcinoma of a T/+ 129/terSV mouse. The first type of cell line was enriched from the original teratocarcinoma cell population by subculture on feeder layers. Its cells morphologically resemble embryonal carcinoma cells; they are highly tumorigenic and can grow either as an ascites or a solid tumor. These cells share antigenic specificities with F9 and PCC4 cells, and do not appear to express T-specific antigens. The second cell type originated from a tumor induced by a mixture of cells which grew out in culture from the original teratocarcinoma. The tumor contained neuroepithelial and embryonal carcinoma cells. Cells of the second line resemble glial cells, and are not killed by anti-F9 antiserum. Cells of the third type were derived from the original teratocarcinoma cell population by subcloning, in the absence of feeder layers. They resemble fibroblasts and are not tumorigenic. They are not killed by anti-F9 antiserum, but do evoke a strong immunological response in syngeneic animals. Antiserum raised against these cells in syngeneic hosts is cytotoxic for three established mouse fibroblast lines but not for mouse fibroblasts from primary and secondary passages, nor fibroblasts of rat, cat and mink origin. These cell lines will be useful for defining new classes of cellular antigens.

577 Nuclear Protein Changes Associated with the in vitro Differentiation of Murine Embryonal Carcinoma Cells to Extraembryonic Endoderm.
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The histone, major non-histone nuclear proteins and average nucleosome repeat distance of cultured murine embryonal carcinoma (ec) cells and extra-embryonic endodermal cells have been compared. The nucleosome repeat distance of two undifferentiated ec cell lines was 196 base pairs of DNA. Primary endodermal cell nuclei isolated from differentiating ec cell cultures had an average size of 205 bp while nuclei from two long term endodermal cell lines had average nucleosone repeats of 187 bp. The core histones of ec and endodermal nuclei appear identical as determined by electrophoresis in either SDS or Triton X-100-acetic acid-urea. However the proportion of the two forms of H1 (H1A and H1B) defined by electrophoresis in SDS varied with the differentiated state. The ratio of H1A/H1B in ec cell nuclei was 1.5-2.0. In contrast endodermal cell nuclei contained less H1A than H1B (H1A/H1B=0.3-0.85). Two abundant nonhistone proteins of approximately 52,000 and 54,000 Daltons were found in nuclei of endodermal cells from long term cell lines, primary endodermal cells isolated from differentiating ec cell cultures and F9 ec cell cultures treated with retinoic acid.These proteins were absent from undifferentiated ec cells. These non-histone nuclear proteins were associated with the nuclear matrix fraction of endodermal nuclei. Both proteins are being purified for immunochemical studies.

578 PURIFICATION OF A CELL SURFACE GLYCOPROTEIN INVOLVED IN THE COMPACTION OF TERATOCARCINOMA CELLS, François Hyafil and François Jacob, Institut Pasteur, Paris, France.

Fab fragments of rabbit IgG raised against the embryonal carcinoma cell line F9, specifically trigger the reversible decompaction of preimplantation embryos (1) as well as of embryonal carcinoma cells (2). We investigated the cell surface molecules involved in this decompaction process. We observed that non-ionic detergent extraction as well as trypsinization of embryonal carcinoma plasma membranes solubilizes molecules capable of specifically inhibiting the decompaction effect of rabbit anti-F9 Fab. The inhibitory activity was purified about 1000 fold from the trypsin extract of membranes and, when analyzed by SDS-PAGE, consisted of a major glycoprotein of MW 84,000 daltons. This 84,000 daltons glycoprotein was specifically immunoprecipitated by rabbit anti-F9 IgG. Further characterization of this glycoprotein will be presented as well as evidence of its involvment in the decompaction of embryonal carcinoma cells by rabbit anti-F9 Fab.

(1) Kemler, R., Babinet, C., Eisen, H. and Jacob, F. (1977) Proc.Nat.Acad.Sci.USA 74, 4449-4452.
(2) Nicolas, J.F., Kemler, R. and Jacob, F. In preparation.

579 SUPRESSION OF TUMORIGENICITY IN TERATOCARCINOMA CYBRIDS, Jerry W. Shay, Gay Lorkowski and Mike A. Clark, The University of Texas Health Science Center, Dallas, TX 75235
It is generally accepted that cybrid clones derived from the fusion of a tumorigenic cytoplasm to a normal cell appear to retain the normal phenotype. However, cybrid clones derived from the fusion of a normal cytoplasm to a tumorigenic cell can either express the tumorigenic phenotype or display a heritable suppression of the tumorigenic phenotype. Using the chloramphenicol resistant cytoplasm from the non-tumorigenic AMT BU A1 cell line [AMT(c)] we have constructed and isolated cybrid clones using two tumorigenic cell lines: the 984 C10 teratocarcinoma cell line which differentiates in cell culture into normal skeletal muscle and makes tumors in nude mice, and the SV_{40}3T3 cell line, which is also highly tumorigenic. The AMT(c)xSV_{40}3T3 cybrids expressed tumorigenicity, and our results are consistent with the previous reports of Zeigler (Som. Cell Gen. 4:477) and Halaban et al. (J.C.B. 83:454a) which suggest that the controlling mechanisms for tumorigenicity reside in the nucleus in cybrids. The AMT(c)x984 C10 cybrids expressed a suppression of the tumorigenic phenotype, and our observations are consistent with the previous reports of Howell and Sager (P.N.A.S. 75:2358) and Coon (J.C.B. 83:449a) which suggest that cybridization can cause heritable changes of the tumorigenic phenotype. It has been suggested that the greatly extended latent period (mean time to produce a tumor) or complete suppression of the tumorigenic phenotype may be correlated with the degree of heteroploid of the cells being used (i.e., near diploid tumorigenic cells can be suppressed, but heteroploid tumorigenic cells cannot). Our results with the heteroploid 984 C10 cybrids are not consistent with this hypothesis and suggest that other factors may be important in determining the tumorigenic phenotype.

580 RETINOIC ACID INDUCES EVERY PC13 EMBRYONAL CARCINOMA CELL TO GIVE RISE TO END CELLS. Vernon C. Bode, Division of Biology, Kansas State University, Manhattan, KS 66506
When injected into adult mice, embryonal carcinoma (EC) cells form tumors containing several cell types. In vitro, some lines can be induced to form embryoid bodies, which resemble the embryonic portion of early egg cylinders, and then differentiate to give several cell types. Under different culture conditions, some EC lines form large flat epithelial-like cells referred to as END cells. Extensive cell growth and death during the time required for the EC → END conversion and cell selection during passage make it difficult to analyze this conversion at the level of individual cells. Specifically, is it a conversion of EC to END cells or do the procedures simply select for existing END cells and the death of EC cells in the population? This report describes the EC → END conversion that is induced in line PC13 clone 5 by retinoic acid. After 48 hrs in media with retinoic acid, the production of plasminogen activator (PA) increases and morphological changes are obvious. The production of PA is used as a biochemical marker of END cells. Cells were plated at low density to give isolated colonies. After two days the established clones were fed with medium containing retinoic acid. At the time of plating, the population had less than 1 PA^+ cell per 1000. When examined five days later, over 90% of the clones exposed to retinoic acid had one or more cells producing PA. Untreated clones (some contained 500 cells) were negative when assayed at the same sensitivity. It is concluded that, although every EC cell is not converted directly to an END cell, essentially every cell in the population can give rise to a PA producing cell and by inference to an END cell.

581 STAGE-SPECIFIC EMBRYONIC ANTIGENS, Lynne Hamburger Shevinsky and Davor Solter, Wistar Institute, Philadelphia, PA 19104
It can be postulated that cell surface interactions regulate differential gene expression during embryonic development. Molecules mediating such interactions can be studied using antisera to stage-specific embryonic antigens. Rats were immunized with 4-8 cell stage mouse embryos and the resulting antiserum was analyzed by complement-mediated cytotoxicity, indirect immunofluorescence, and radioimmunoassay. Antiserum reactivity can be detected starting at the 8 cell stage, continuing until implantation. The antiserum does not appear to react with any other mouse cell, including teratocarcinoma. Monoclonal antibody has been obtained by fusing the immunized rats spleen with mouse myeloma cells, and it's reactivity appears to be the same as that of the complex antiserum. A second antiserum was obtained by immunizing mice with a mouse-human SV-40 transformed hybrid cell line. This antiserum reacts against all stages of preimplantation mouse embryos, with greatest reactivity at the 4-8 cell stage. The antigen(s) was also detected on mouse teratocarcinoma cells and some mouse SV-40 transformed cell lines. The antigen(s) was not found on SV-40 transformed cell lines from other species nor on various human and mouse cell lines. This antiserum appears to detect an antigen common to mouse embryo, teratocarcinoma cells and some mouse SV-40 transformed cells lines, suggesting that the antigen may be embryonic in nature, and exposed in the differentiated cell type as a consequence of SV-40 transformation.

582 CHARACTERIZATION OF PLURIPOTENT MUTANT MOUSE TERATOCARCINOMA CELLS, David W. Martin, Jr., Lorraine J. Gudas, and Barbara Levinson, University of California, San Francisco, San Francisco, CA 94143.
For the purpose of generating chimeric animals with enzyme deficient tissues and ultimately for providing animal models of specific human genetic diseases, we have employed the technique of Gardner for introducing teratocarcinomas into mouse blastocysts. We have isolated a variety of mutants in the teratocarcinoma cell line PSA-1, a pluripotent, feeder-dependent line derived from a strain 129 mouse embryo. PSA-1 cells were mutagenized in vitro with N-methyl-N-nitro nitroso-guanidine (MNNG) and then were selected in arabinosyl cytosine (araC), cordycepin, or bromodeoxyuridine (BrdUrd). Selective clonings were done either in liquid medium directly on a feeder layer of mouse embryo fibroblasts or within 0.35% agarose with an agarose layer between the PSA-1 cells and the feeder layer. Mutants deficient in deoxycytidine kinase activity were obtained from the araC selection, mutants deficient in adenosine kinase activity were obtained in cordycepin, and two types of mutants resulted from the selection in BrdUrd. One mutant had 30% of wild type thymidine kinase (TK) activity, whereas a second mutant, BUdr-40-4, had <2% of wild type TK activity. The BUdr-40-4 cells are killed in HAT medium, and they are more than 1,000-fold less sensitive to BrdUrd than are wild type cells. When BUdr-40-4 cells are injected subcutaneously into a 129 mouse, they produce tumors containing a multitude of tissue types, typical of teratomas, despite exposure to the chemical mutagen MNNG. We are currently injecting these BUdr-40-4 cells into 3½ day C57Bl/6 mouse blastocysts and transferring the manipulated blastocysts into pseudopregnant female mice for the generation of chimeric animals.

583 RESTRICTION OF MURINE RETROVIRUSES IN TERATOCARCINOMA CELLS: PROVIRAL DNA STUDIES. W.K. Yang[1], L. d'Auriol[2], D.M. Yang[1], J.O. Kiggans[1], C.Y. Ou[1], J. Peries[2], and R. Emanoil-Ravicovitch[2], Biol. Div. Oak Ridge Nat'l. Lab., Oak Ridge, TN 37830 USA[1] and U. INSERM 107 Virologie des Leucémies, Hôpital Saint Louis, Paris, France[2].
Infectivity of retroviruses in cultured teratocarcinoma cells has been found to be affected by the state of cellular differentiation. The undifferentiated cell lines are usually resistant to the infection. To study this restriction phenomenon, we have employed the agarose electrophoresis/DBM-paper transfer/hybridization method as well as the DNA transfection procedure for the analysis of proviral DNA formation in two teratocarcinoma cell lines (PCC4 and PCD1) infected with an N-tropic retrovirus. After inoculation of the virus, formation of proviral DNA was detected in both PCD1 and PCC4 cells-a sequential appearance and decline of the form III DNA (from 4 to 18 hr) and the two form I DNAs (from 8 to 24 hr). A secondary burst of proviral DNA formation, however, was observed in PCD1 cells but not in PCC4 cells. The unintegrated proviral DNA preparations isolated from both cells 10 hr after virus inoculation were found to be infectious in a DNA transfection assay. Both lines of teratocarcinoma cells were hardly competent when tested with infectious DNA preparations containing N-tropic virus genome. These results suggest that retrovirus replication in the undifferentiated teratocarcinoma cells is blocked at a step beyond the proviral DNA synthesis and maturation. Further studies on the integration of proviral DNA in these cells are in progress. (Research sponsored jointly by NCI under Interagency Agreement YO1-CP6-0500 and the OHER, U.S.DOE, under contract W-7405-eng-26 with the Union Carbide Corporation.

584 TERATOCARCINOMA STEM CELLS LACK A NUCLEAR FUNCTION REQUIRED FOR VIRUS REPLICATION, James W. Gautsch, Scripps Clinic and Research Foundation, La Jolla, CA 92037
A fundamental difference between undifferentiated embryonal carcinoma (EC) cells and differentiated successors is manifest as the total restriction of MuLV growth in EC cells. The EC cell block to virus growth occurs during an intracellular, post-integration step in the life cycle of MuLV (Teich, et al Cell, 12, 973, 1977). The studies reported here show that fusion of MuLV-infected EC cells with differentiated mouse, mink, or rabbit cells results in virus production. Therefore the block to virus growth is a recessive trait of EC cells and is due to the lack of a host cell function(s) vital to virus replication, but not necessary to cell growth. Heterokaryon formation 8 to 12 hours after infection of EC cells results in optimum virus production. This suggested that 1) the complementing function supplied by the permissive cell was labile and had a half-life of approximately four hours, 2) production of the complementing function in heterokaryons was repressed, and 3) the virus could utilize the function for growth only during a specific portion of its replication cycle. Fusion of infected EC cells with permissive cell cytoplasts did not complement MuLV growth. However, fusion of infected EC cells with permissive cell karyoplasts complemented MuLV growth as efficiently as whole cells. These results suggest that a host cell function present in nuclei of differentiated cells is required for MuLV replication, and that expression of this function is repressed in undifferentiated EC cells. If the complementation of MuLV growth in EC cells can be used to purify the responsible factor(s) from permissive cell nuclei, the related function(s) should become clear.

585 INDEPENDENT REGULATION OF H-2K AND H-2D GENE EXPRESSION IN MURINE TERATOCARCINOMA SOMATIC CELL HYBRIDS, Davor Solter, Rudolf Gmur and Barbara B. Knowles, Wistar Institute, Philadelphia, PA 19104
We have obtained results suggesting that there is independent regulation of expression of the H-2K and H-2D gene products. These results are based on analysis of somatic cell hybrids derived from fusion of two different teratocarcinoma stem cell lines (PCC4 aza 1 and F9 BUdR) with normal mouse spleen cells and a mouse thymoma derived cell line (BW 5147) respectively. Isolated hybrid clones were tested for the H-2K and H-2D gene expression by quantitative absorption and cytotoxicity and by indirect antibody binding radioimmunoassay. Somatic cell hybrids between PCC4 aza 1 and spleen cells are phenotypically identical to teratocarcinoma stem cells and do not express H-2 antigens. However after differentiation in vitro one hybrid expresses H-2 antigens of both parental cell lines, one expresses H-2D coded antigens but not H-2K antigenic specificities and one does not express any H-2 antigens. Somatic cell hybrids between F9 BUdR and BW 5147 resemble fibroblasts. These hybrid clones can be divided into those which express the H-2K and H-2D gene products of both parental alleles, those which express H-2D specificities strongly and H-2K antigens very weakly, those which express H-2D but not H-2K gene products and those which do not express H-2 at all. These results suggest complex regulatory mechanisms which are H-2K and H-2D (and probably allele) specific.